



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification<sup>6</sup> :</b> C12N 15/90, 15/85, C12Q 1/68, C12N 5/10, 9/12, 15/13, C07K 16/28, C12N 15/12, C07K 14/705, G01N 33/53, C12N 15/62, C07K 19/00	<b>A1</b>	<b>(11) International Publication Number:</b> WO 98/41645 <b>(43) International Publication Date:</b> 24 September 1998 (24.09.98)
<b>(21) International Application Number:</b> PCT/US98/03935 <b>(22) International Filing Date:</b> 9 March 1998 (09.03.98)  <b>(30) Priority Data:</b> 08/819,866 14 March 1997 (14.03.97) US 09/023,715 13 February 1998 (13.02.98) US  <b>(71) Applicant:</b> IDEC PHARMACEUTICALS CORPORATION [US/US]; 11011 Torreyana Road, San Diego, CA 92121 (US).  <b>(72) Inventors:</b> REFF, Mitchell, E.; 4166 Combe Way, San Diego, CA 92122 (US). BARNETT, Richard, Spence; 306 Belmont Court, San Marcos, CA 92069 (US). McLACHLAN, Karen, Retta; Apartment B6, 766 South Nardo, Solana Beach, CA 92075 (US).  <b>(74) Agents:</b> GESS, E., Joseph et al.; Burns, Doane, Swecker & Mathis L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(54) Title:</b> METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME		
<b>(57) Abstract</b>  A method for achieving site specific integration of a desired DNA at a target site in a mammalian cell via homologous recombination is described. This method provides for the reproducible selection of cell lines wherein a desired DNA is integrated at a predetermined transcriptionally active site previously marked with a marker plasmid. The method is particularly suitable for the production of mammalian cell lines which secrete mammalian proteins at high levels, in particular immunoglobulins. Vectors and vector combinations for use in the subject cloning method are also provided.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

### Title of the Invention

METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA  
HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME

5

### Field of the Invention

The present invention relates to a process of targeting the integration of a desired exogenous DNA to a specific location within the genome of a mammalian cell.

10 More specifically, the invention describes a novel method for identifying a transcriptionally active target site ("hot spot") in the mammalian genome, and inserting a desired DNA at this site via homologous recombination.

15 The invention also optionally provides the ability for gene amplification of the desired DNA at this location by co-integrating an amplifiable selectable marker, e.g., DHFR, in combination with the exogenous DNA. The invention additionally describes the construction of novel vectors suitable for accomplishing the above, and

20 further provides mammalian cell lines produced by such methods which contain a desired exogenous DNA integrated at a target hot spot.

- 2 -

Background

Technology for expressing recombinant proteins in both prokaryotic and eukaryotic organisms is well established. Mammalian cells offer significant advantages over bacteria or yeast for protein production, resulting from their ability to correctly assemble, glycosylate and post-translationally modify recombinantly expressed proteins. After transfection into the host cells, recombinant expression constructs can be maintained as extrachromosomal elements, or may be integrated into the host cell genome. Generation of stably transfected mammalian cell lines usually involves the latter; a DNA construct encoding a gene of interest along with a drug resistance gene (dominant selectable marker) is introduced into the host cell, and subsequent growth in the presence of the drug allows for the selection of cells that have successfully integrated the exogenous DNA. In many instances, the gene of interest is linked to a drug resistant selectable marker which can later be subjected to gene amplification. The gene encoding dihydrofolate reductase (DHFR) is most commonly used for this purpose. Growth of cells in the presence of methotrexate, a competitive inhibitor of DHFR, leads to increased DHFR production by means of amplification of the DHFR gene. As flanking regions of DNA will also become amplified, the resultant coamplification of a DHFR linked gene in the transfected cell line can lead to increased protein

- 3 -

production, thereby resulting in high level expression of the gene of interest.

While this approach has proven successful, there are a number of problems with the system because of the random nature of the integration event. These problems exist because expression levels are greatly influenced by the effects of the local genetic environment at the gene locus, a phenomena well documented in the literature and generally referred to as "position effects" (for example, see Al-Shawi et al, *Mol. Cell. Biol.*, 10:1192-1198 (1990); Yoshimura et al, *Mol. Cell. Biol.*, 7:1296-1299 (1987)). As the vast majority of mammalian DNA is in a transcriptionally inactive state, random integration methods offer no control over the transcriptional fate of the integrated DNA. Consequently, wide variations in the expression level of integrated genes can occur, depending on the site of integration. For example, integration of exogenous DNA into inactive, or transcriptionally "silent" regions of the genome will result in little or no expression. By contrast integration into a transcriptionally active site may result in high expression.

Therefore, when the goal of the work is to obtain a high level of gene expression, as is typically the desired outcome of genetic engineering methods, it is generally necessary to screen large numbers of transfectants to find such a high producing clone.

- 4 -

Additionally, random integration of exogenous DNA into the genome can in some instances disrupt important cellular genes, resulting in an altered phenotype. These factors can make the generation of high expressing stable mammalian cell lines a complicated and laborious process.

Recently, our laboratory has described the use of DNA vectors containing translationally impaired dominant selectable markers in mammalian gene expression. (This is disclosed in U.S. Serial No. 08/147,696 filed November 3, 1993, recently allowed).

These vectors contain a translationally impaired neomycin phosphotransferase (neo) gene as the dominant selectable marker, artificially engineered to contain an intron into which a DHFR gene along with a gene or genes of interest is inserted. Use of these vectors as expression constructs has been found to significantly reduce the total number of drug resistant colonies produced, thereby facilitating the screening procedure in relation to conventional mammalian expression vectors. Furthermore, a significant percentage of the clones obtained using this system are high expressing clones. These results are apparently attributable to the modifications made to the neo selectable marker. Due to the translational impairment of the neo gene, transfected cells will not produce enough neo protein to survive drug selection, thereby decreasing the overall

- 5 -

number of drug resistant colonies. Additionally, a higher percentage of the surviving clones will contain the expression vector integrated into sites in the genome where basal transcription levels are high, resulting in overproduction of neo, thereby allowing the cells to overcome the impairment of the neo gene. Concomitantly, the genes of interest linked to neo will be subject to similar elevated levels of transcription. This same advantage is also true as a result of the artificial intron created within neo; survival is dependent on the synthesis of a functional neo gene, which is in turn dependent on correct and efficient splicing of the neo introns. Moreover, these criteria are more likely to be met if the vector DNA has integrated into a region which is already highly transcriptionally active.

Following integration of the vector into a transcriptionally active region, gene amplification is performed by selection for the DHFR gene. Using this system, it has been possible to obtain clones selected using low levels of methotrexate (50nM), containing few (<10) copies of the vector which secrete high levels of protein (>55pg/cell/day). Furthermore, this can be achieved in a relatively short period of time. However, the success in amplification is variable. Some transcriptionally active sites cannot be amplified and

- 6 -

therefore the frequency and extent of amplification from a particular site is not predictable.

Overall, the use of these translationally impaired vectors represents a significant improvement over other  
5 methods of random integration. However, as discussed, the problem of lack of control over the integration site remains a significant concern.

One approach to overcome the problems of random integration is by means of gene targeting, whereby the  
10 exogenous DNA is directed to a specific locus within the host genome. The exogenous DNA is inserted by means of homologous recombination occurring between sequences of DNA in the expression vector and the corresponding homologous sequence in the genome. However, while this  
15 type of recombination occurs at a high frequency naturally in yeast and other fungal organisms, in higher eukaryotic organisms it is an extremely rare event. In mammalian cells, the frequency of homologous versus non-homologous (random integration) recombination is reported  
20 to range from 1/100 to 1/5000 (for example, see Capecchi, *Science*, 244:1288-1292 (1989); Morrow and Kucherlapati, *Curr. Op. Biotech.*, 4:577-582 (1993)).

One of the earliest reports describing homologous recombination in mammalian cells comprised an artificial  
25 system created in mouse fibroblasts (Thomas et al, *Cell*, 44:419-428 (1986)). A cell line containing a mutated, non-functional version of the neo gene integrated into

- 7 -

the host genome was created, and subsequently targeted with a second non-functional copy of neo containing a different mutation. Reconstruction of a functional neo gene could occur only by gene targeting. Homologous recombination 5 recombinants were identified by selecting for G418 resistant cells, and confirmed by analysis of genomic DNA isolated from the resistant clones.

Recently, the use of homologous recombination to replace the heavy and light immunoglobulin genes at endogenous loci in antibody secreting cells has been reported. (U.S. Patent No. 5,202,238, Fell et al, (1993).) However, this particular approach is not widely applicable, because it is limited to the production of immunoglobulins in cells which 15 endogenously express immunoglobulins, e.g., B cells and myeloma cells. Also, expression is limited to single copy gene levels because co-amplification after homologous recombination is not included. The method is further complicated by the fact that two separate 20 integration events are required to produce a functional immunoglobulin: one for the light chain gene followed by one for the heavy chain gene.

An additional example of this type of system has been reported in NS/O cells, where recombinant 25 immunoglobulins are expressed by homologous recombination into the immunoglobulin gamma 2A locus (Hollis et al, international patent application #

- 8 -

PCT/IB95 (00014).) Expression levels obtained from this site were extremely high - on the order of 20pg/cell/day from a single copy integrant. However, as in the above example, expression is limited to this level because an  
5 amplifiable gene is not contegrated in this system. Also, other researchers have reported aberrant glycosylation of recombinant proteins expressed in NS/O cells (for example, see Flesher et al, *Biotech. and Bioeng.*, 48:399-407 (1995)), thereby limiting the  
10 applicability of this approach.

The cre-loxP recombination system from bacteriophage P1 has recently been adapted and used as a means of gene targeting in eukaryotic cells: Specifically, the site specific integration of exogenous  
15 DNA into the Chinese hamster ovary (CHO) cell genome using cre recombinase and a series of lox containing vectors have been described. (Fukushige and Sauer, *Proc. Natl. Acad. Sci. USA*, 89:7905-7909 (1992).) This system is attractive in that it provides for  
20 reproducible expression at the same chromosomal location. However, no effort was made to identify a chromosomal site from which gene expression is optimal, and as in the above example, expression is limited to single copy levels in this system. Also, it is  
25 complicated by the fact that one needs to provide for expression of a functional recombinase enzyme in the mammalian cell.

- 9 -

The use of homologous recombination between an introduced DNA sequence and its endogenous chromosomal locus has also been reported to provide a useful means of genetic manipulation in mammalian cells, as well as in yeast cells. (See e.g., Bradley et al, *Meth. Enzymol.*, 223:855-879 (1993); Capecchi, *Science*, 244:1288-1292 (1989); Rothstein et al, *Meth. Enzymol.*, 194:281-301 (1991)). To date, most mammalian gene targeting studies have been directed toward gene disruption ("knockout") or site-specific mutagenesis of selected target gene loci in mouse embryonic stem (ES) cells. The creation of these "knockout" mouse models has enabled scientists to examine specific structure-function issues and examine the biological importance of a myriad of mouse genes. This field of research also has important implications in terms of potential gene therapy applications.

Also, vectors have recently been reported by Celltech (Kent, U.K.) which purportedly are targeted to transcriptionally active sites in NSO cells, which do not require gene amplification (Peakman et al, *Hum. Antibod. Hybridomas*, 5:65-74 (1994)). However, levels of immunoglobulin secretion in these unamplified cells have not been reported to exceed 20pg/cell/day, while in amplified CHO cells, levels as high as 100pg/cell/day can be obtained (*Id.*).

- 10 -

It would be highly desirable to develop a gene targeting system which reproducibly provided for the integration of exogenous DNA into a predetermined site in the genome known to be transcriptionally active.

5 Also, it would be desirable if such a gene targeting system would further facilitate co-amplification of the inserted DNA after integration. The design of such a system would allow for the reproducible and high level expression of any cloned gene of interest in a mammalian  
10 cell, and undoubtedly would be of significant interest to many researchers.

In this application, we provide a novel mammalian expression system, based on homologous recombination occurring between two artificial substrates contained in  
15 two different vectors. Specifically, this system uses a combination of two novel mammalian expression vectors, referred to as a "marking" vector and a "targeting" vector.

Essentially, the marking vector enables the identification and marking of a site in the mammalian genome  
20 which is transcriptionally active, i.e., a site at which gene expression levels are high. This site can be regarded as a "hot spot" in the genome. After integration of the marking vector, the subject expression system enables another DNA to be integrated at this site,  
25 i.e., the targeting vector, by means of homologous recombination occurring between DNA sequences common to

- 11 -

both vectors. This system affords significant advantages over other homologous recombination systems.

Unlike most other homologous systems employed in mammalian cells, this system exhibits no background.

5 Therefore, cells which have only undergone random integration of the vector do not survive the selection.

Thus, any gene of interest cloned into the targeting plasmid is expressed at high levels from the marked hot spot. Accordingly, the subject method of gene expres-

10 sion substantially or completely eliminates the problems inherent to systems of random integration, discussed in detail above. Moreover, this system provides reproduc-

ible and high level expression of any recombinant protein at the same transcriptionally active site in the

15 mammalian genome. In addition, gene amplification may be effected at this particular transcriptionally active site by including an amplifiable dominant selectable marker (e.g. DHFR) as part of the marking vector.

#### Objects of the Invention

20 Thus, it is an object of the invention to provide an improved method for targeting a desired DNA to a specific site in a mammalian cell.

It is a more specific object of the invention to provide a novel method for targeting a desired DNA to a  
25 specific site in a mammalian cell via homologous recombination.

- 12 -

It is another specific object of the invention to provide novel vectors for achieving site specific integration of a desired DNA in a mammalian cell.

It is still another object of the invention to  
5 provide novel mammalian cell lines which contain a desired DNA integrated at a predetermined site which provides for high expression.

It is a more specific object of the invention to provide a novel method for achieving site specific integration of a desired DNA in a Chinese hamster ovary  
10 (CHO) cell.

It is another more specific object of the invention to provide a novel method for integrating immunoglobulin genes, or any other genes, in mammalian cells at  
15 predetermined chromosomal sites that provide for high expression.

It is another specific object of the invention to provide novel vectors and vector combinations suitable for integrating immunoglobulin genes into mammalian  
20 cells at predetermined sites that provide for high expression.

It is another object of the invention to provide mammalian cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high  
25 expression.

It is an even more specific object of the invention to provide a novel method for integrating immunoglobulin

- 13 -

genes into CHO cells that provide for high expression, as well as novel vectors and vector combinations that provide for such integration of immunoglobulin genes into CHO cells.

5        In addition, it is a specific object of the invention to provide novel CHO cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high expression, and have been amplified by methotrexate selection to secrete even greater amounts  
10       of functional immunoglobulins.

#### Brief Description of the Figures

Figure 1 depicts a map of a marking plasmid according to the invention referred to as Desmond. The plasmid is shown in circular form (1a) as well as a  
15       linearized version used for transfection (1b).

Figure 2(a) shows a map of a targeting plasmid referred to "Molly". Molly is shown here encoding the anti-CD20 immunoglobulin genes, expression of which is described in Example 1.

20       Figure 2(b) shows a linearized version of Molly, after digestion with the restriction enzymes KpnI and PacI. This linearized form was used for transfection.

Figure 3 depicts the potential alignment between Desmond sequences integrated into the CHO genome, and  
25       incoming targeting Molly sequences. One potential ar-

- 14 -

rangement of Molly integrated into Desmond after homologous recombination is also presented.

Figure 4 shows a Southern analysis of single copy Desmond clones. Samples are as follows:

- 5 Lane 1:  $\lambda$ HindIII DNA size marker
- Lane 2: Desmond clone 10F3
- Lane 3: Desmond clone 10C12
- Lane 4: Desmond clone 15C9
- Lane 5: Desmond clone 14B5
- 10 Lane 6: Desmond clone 9B2

Figure 5 shows a Northern analysis of single copy Desmond clones. Samples are as follows: Panel A: northern probed with CAD and DHFR probes, as indicated on the figure. Panel B: duplicate northern, probed with  
15 CAD and HisD probes, as indicated. The RNA samples loaded in panels A and B are as follows:

- Lane 1: clone 9B2, lane 2; clone 10C12, lane 3; clone 14B5, lane 4; clone 15C9, lane 5; control RNA from CHO transfected with a HisD and DHFR containing plasmid,  
20 lane 6; untransfected CHO.

Figure 6 shows a Southern analysis of clones resulting from the homologous integration of Molly into Desmond. Samples are as follows:

- Lane 1:  $\lambda$ HindIII DNA size markers, Lane 2: 20F4, lane 3;  
25 5F9, lane 4; 21C7, lane 5; 24G2, lane 6; 25E1, lane 7; 28C9, lane 8; 29F9, lane 9; 39G11, lane 10; 42F9, lane 11; 50G10, lane 12; Molly plasmid DNA, linearized with

- 15 -

BglIII(top band) and cut with BglIII and KpnI (lower band), lane 13; untransfected Desmond.

Figures 7A through 7G contain the Sequence Listing for Desmond.

5       Figures 8A through 8I contain the Sequence Listing for Molly-containing anti-CD20.

Figure 9 contains a map of the targeting plasmid, "Mandy," shown here encoding anti-CD23 genes, the expression of which is disclosed in Example 5.

10       Figures 10A through 10N contain the sequence listing of "Mandy" containing the anti-CD23 genes as disclosed in Example 5.

#### Detailed Description of the Invention

15       The invention provides a novel method for integrating a desired exogenous DNA at a target site within the genome of a mammalian cell via homologous recombination. Also, the invention provides novel vectors for achieving the site specific integration of a DNA at a target site in the genome of a mammalian cell.

20       More specifically, the subject cloning method provides for site specific integration of a desired DNA in a mammalian cell by transfection of such cell with a "marker plasmid" which contains a unique sequence that is foreign to the mammalian cell genome and which  
25       provides a substrate for homologous recombination, followed by transfection with a "target plasmid" containing

- 16 -

a sequence which provides for homologous recombination with the unique sequence contained in the marker plasmid, and further comprising a desired DNA that is to be integrated into the mammalian cell. Typically, the integrated DNA will encode a protein of interest, such as an immunoglobulin or other secreted mammalian glycoprotein.

The exemplified homologous recombination system uses the neomycin phosphotransferase gene as a dominant selectable marker. This particular marker was utilized based on the following previously published observations;

(i) the demonstrated ability to target and restore function to a mutated version of the neo gene (cited earlier) and

(ii) our development of translationally impaired expression vectors, in which the neo gene has been artificially created as two exons with a gene of interest inserted in the intervening intron; neo exons are correctly spliced and translated in vivo, producing a functional protein and thereby conferring G418 resistance on the resultant cell population. In this application, the neo gene is split into three exons. The third exon of neo is present on the "marker" plasmid and becomes integrated into the host cell genome upon integration of the marker plasmid into the mammalian cells. Exons 1 and 2 are present on the targeting plasmid, and are separated

- 17 -

by an intervening intron into which at least one gene of interest is cloned. Homologous recombination of the targeting vector with the integrated marking vector results in correct splicing of all three exons of the neo gene and thereby expression of a functional neo protein (as determined by selection for G418 resistant colonies). Prior to designing the current expression system, we had experimentally tested the functionality of such a triply spliced neo construct in mammalian cells. The results of this control experiment indicated that all three neo exons were properly spliced and therefore suggested the feasibility of the subject invention.

However, while the present invention is exemplified using the neo gene, and more specifically a triple split neo gene, the general methodology should be efficacious with other dominant selectable markers.

As discussed in greater detail *infra*, the present invention affords numerous advantages to conventional gene expression methods, including both random integration and gene targeting methods. Specifically, the subject invention provides a method which reproducibly allows for site-specific integration of a desired DNA into a transcriptionally active domain of a mammalian cell. Moreover, because the subject method introduces an artificial region of "homology" which acts as a unique substrate for homologous recombination and the

- 18 -

insertion of a desired DNA, the efficacy of subject invention does not require that the cell endogenously contain or express a specific DNA. Thus, the method is generically applicable to all mammalian cells, and can  
5 be used to express any type of recombinant protein.

The use of a triply spliced selectable marker, e.g., the exemplified triply spliced neo construct, guarantees that all G418 resistant colonies produced will arise from a homologous recombination event (random  
10 integrants will not produce a functional neo gene and consequently will not survive G418 selection). Thus, the subject invention makes it easy to screen for the desired homologous event. Furthermore, the frequency of additional random integrations in a cell that has under-  
15 gone a homologous recombination event appears to be low.

Based on the foregoing, it is apparent that a significant advantage of the invention is that it substantially reduces the number of colonies that need be screened to identify high producer clones, i.e., cell  
20 lines containing a desired DNA which secrete the corresponding protein at high levels. On average, clones containing integrated desired DNA may be identified by screening about 5 to 20 colonies (compared to several thousand which must be screened when using standard  
25 random integration techniques, or several hundred using the previously described intronic insertion vectors) Additionally, as the site of integration was preselected

- 19 -

and comprises a transcriptionally active domain, all exogenous DNA expressed at this site should produce comparable, i.e. high levels of the protein of interest.

Moreover, the subject invention is further advantageous in that it enables an amplifiable gene to be inserted on integration of the marking vector. Thus, when a desired gene is targeted to this site via homologous recombination, the subject invention allows for expression of the gene to be further enhanced by gene amplification. In this regard, it has been reported in from the literature that different genomic sites have different capacities for gene amplification (Meinkoth et al, *Mol. Cell Biol.*, 7:1415-1424 (1987)). Therefore, this technique is further advantageous as it allows for the placement of a desired gene of interest at a specific site that is both transcriptionally active and easily amplified. Therefore, this should significantly reduce the amount of time required to isolate such high producers.

Specifically, while conventional methods for the construction of high expressing mammalian cell lines can take 6 to 9 months, the present invention allows for such clones to be isolated on average after only about 3-6 months. This is due to the fact that conventionally isolated clones typically must be subjected to at least three rounds of drug resistant gene amplification in order to reach satisfactory levels of gene expression.

- 20 -

As the homologously produced clones are generated from a preselected site which is a high expression site, fewer rounds of amplification should be required before reaching a satisfactory level of production.

5        Still further, the subject invention enables the reproducible selection of high producer clones wherein the vector is integrated at low copy number, typically single copy. This is advantageous as it enhances the stability of the clones and avoids other potential adverse side-effects associated with high copy number. As  
10        described *supra*, the subject homologous recombination system uses the combination of a "marker plasmid" and a "targeting plasmid" which are described in more detail below.

15        The "marker plasmid" which is used to mark and identify a transcriptionally hot spot will comprise at least the following sequences:

         (i) a region of DNA that is heterologous or unique to the genome of the mammalian cell, which functions as  
20        a source of homology, allows for homologous recombination (with a DNA contained in a second target plasmid). More specifically, the unique region of DNA (i) will generally comprise a bacterial, viral, yeast synthetic, or other DNA which is not normally present in the  
25        mammalian cell genome and which further does not comprise significant homology or sequence identity to DNA contained in the genome of the mammalian cell.

- 21 -

Essentially, this sequence should be sufficiently different to mammalian DNA that it will not significantly recombine with the host cell genome via homologous recombination. The size of such unique DNA will generally be at least about 2 to 10 kilobases in size, or higher, more preferably at least about 10kb, as several other investigators have noted an increased frequency of targeted recombination as the size of the homology region is increased (Capecchi, *Science*, 244:1288-1292 (1989)).

The upper size limit of the unique DNA which acts as a site for homologous recombination with a sequence in the second target vector is largely dictated by potential stability constraints (if DNA is too large it may not be easily integrated into a chromosome and the difficulties in working with very large DNAs.

(ii) a DNA including a fragment of a selectable marker DNA, typically an exon of a dominant selectable marker gene. The only essential feature of this DNA is that it not encode a functional selectable marker protein unless it is expressed in association with a sequence contained in the target plasmid. Typically, the target plasmid will comprise the remaining exons of the dominant selectable marker gene (those not comprised in "targeting" plasmid). Essentially, a functional selectable marker should only be produced if homologous recombination occurs (resulting in the association and

- 22 -

expression of this marker DNA (i) sequence together with the portion(s) of the selectable marker DNA fragment which is (are) contained in the target plasmid).

As noted, the current invention exemplifies the use of the neomycin phosphotransferase gene as the dominant selectable marker which is "split" in the two vectors. However, other selectable markers should also be suitable, e.g., the Salmonella histidinol dehydrogenase gene, hygromycin phosphotransferase gene, herpes simplex virus thymidine kinase gene, adenosine deaminase gene, glutamine synthetase gene and hypoxanthine-guanine phosphoribosyl transferase gene.

(iii) a DNA which encodes a functional selectable marker protein, which selectable marker is different from the selectable marker DNA (ii). This selectable marker provides for the successful selection of mammalian cells wherein the marker plasmid is successfully integrated into the cellular DNA. More preferably, it is desirable that the marker plasmid comprise two such dominant selectable marker DNAs, situated at opposite ends of the vector. This is advantageous as it enables integrants to be selected using different selection agents and further enables cells which contain the entire vector to be selected. Additionally, one marker can be an amplifiable marker to facilitate gene amplification as discussed previously. Any of the

- 23 -

dominant selectable marker listed in (ii) can be used as well as others generally known in the art.

Moreover, the marker plasmid may optionally further comprise a rare endonuclease restriction site. This is  
5 potentially desirable as this may facilitate cleavage.

If present, such rare restriction site should be situated close to the middle of the unique region that acts as a substrate for homologous recombination. Preferably such sequence will be at least about 12 nucleotides.

10 The introduction of a double stranded break by similar methodology has been reported to enhance the frequency of homologous recombination. (Choulika et al, *Mol. Cell. Biol.*, 15:1968-1973 (1995)). However, the presence of such sequence is not essential.

15 The "targeting plasmid" will comprise at least the following sequences:

(1) the same unique region of DNA contained in the marker plasmid or one having sufficient homology or sequence identity therewith that said DNA is capable of  
20 combining via homologous recombination with the unique region (i) in the marker plasmid. Suitable types of DNAs are described *supra* in the description of the unique region of DNA (1) in the marker plasmid.

(2) The remaining exons of the dominant selectable  
25 marker, one exon of which is included as (ii) in the marker plasmid listed above. The essential features of this DNA fragment is that it result in a functional

- 24 -

(selectable) marker protein only if the target plasmid integrates via homologous recombination (wherein such recombination results in the association of this DNA with the other fragment of the selectable marker DNA contained in the marker plasmid) and further that it allow for insertion of a desired exogenous DNA. Typically, this DNA will comprise the remaining exons of the selectable marker DNA which are separated by an intron. For example, this DNA may comprise the first two exons of the neo gene and the marker plasmid may comprise the third exon (back third of neo).

(3) The target plasmid will also comprise a desired DNA, e.g., one encoding a desired polypeptide, preferably inserted within the selectable marker DNA fragment contained in the plasmid. Typically, the DNA will be inserted in an intron which is comprised between the exons of the selectable marker DNA. This ensures that the desired DNA is also integrated if homologous recombination of the target plasmid and the marker plasmid occurs. This intron may be naturally occurring or it may be engineered into the dominant selectable marker DNA fragment.

This DNA will encode any desired protein, preferably one having pharmaceutical or other desirable properties. Most typically the DNA will encode a mammalian protein, and in the current examples provided, an immunoglobulin or an immunoadhesin. However the

- 25 -

invention is not in any way limited to the production of immunoglobulins.

As discussed previously, the subject cloning method is suitable for any mammalian cell as it does not require for efficacy that any specific mammalian sequence or sequences be present. In general, such mammalian cells will comprise those typically used for protein expression, e.g., CHO cells, myeloma cells, COS cells, BHK cells, Sp2/0 cells, NIH 3T3 and HeLa cells. In the examples which follow, CHO cells were utilized. The advantages thereof include the availability of suitable growth medium, their ability to grow efficiently and to high density in culture, and their ability to express mammalian proteins such as immunoglobulins in biologically active form.

Further, CHO cells were selected in large part because of previous usage of such cells by the inventors for the expression of immunoglobulins (using the translationally impaired dominant selectable marker containing vectors described previously). Thus, the present laboratory has considerable experience in using such cells for expression. However, based on the examples which follow, it is reasonable to expect similar results will be obtained with other mammalian cells.

In general, transformation or transfection of mammalian cells according to the subject invention will be effected according to conventional methods. So that the

- 26 -

invention may be better understood, the construction of exemplary vectors and their usage in producing integrants is described in the examples below.

#### EXAMPLE 1

5

##### Design and Preparation of Marker and Targeting Plasmid DNA Vectors

The marker plasmid herein referred to as "Desmond" was assembled from the following DNA elements:

(a) Murine dihydrofolate reductase gene (DHFR),  
10 incorporated into a transcription cassette, comprising the mouse beta globin promoter 5" to the DHFR start site, and bovine growth hormone poly adenylation signal 3" to the stop codon. The DHFR transcriptional cassette was isolated from TCAE6, an expression vector created  
15 previously in this laboratory (Newman et al, 1992, *Bio-technology*, 10:1455-1460).

(b) E. coli  $\beta$ -galactosidase gene - commercially available, obtained from Promega as pSV-b-galactosidase control vector, catalog # E1081.

20 (c) Baculovirus DNA, commercially available, purchased from Clontech as pBAKPAK8, cat # 6145-1.

(d) Cassette comprising promoter and enhancer elements from Cytomegalovirus and SV40 virus. The cassette was generated by PCR using a derivative of expression  
25 vector TCAE8 (Reff et al, *Blood*, 83:435-445 (1994)). The enhancer cassette was inserted within the baculo-

- 27 -

virus sequence, which was first modified by the insertion of a multiple cloning site.

(e) E. coli GUS (glucuronidase) gene, commercially available, purchased from Clontech as pB101, cat. #  
5 6017-1.

(f) Firefly luciferase gene, commercially available, obtained from Promega as pGEM-Luc (catalog # E1541).

(g) S. typhimurium histidinol dehydrogenase gene  
10 (HisD). This gene was originally a gift from (Donahue et al, Gene, 18:47-59 (1982)), and has subsequently been incorporated into a transcription cassette comprising the mouse beta globin major promoter 5' to the gene, and the SV40 polyadenylation signal 3' to the gene.

15 The DNA elements described in (a)-(g) were combined into a pBR derived plasmid backbone to produce a 7.7kb contiguous stretch of DNA referred to in the attached figures as "homology". Homology in this sense refers to  
20 sequences of DNA which are not part of the mammalian genome and are used to promote homologous recombination between transfected plasmids sharing the same homology DNA sequences.

(h) Neomycin phosphotransferase gene from TN5 (Davis and Smith, Ann. Rev. Micro., 32:469-518 (1978)).  
25 The complete neo gene was subcloned into pBluescript SK-(Stratagene catalog # 212205) to facilitate genetic manipulation. A synthetic linker was then inserted into

- 28 -

a unique Pst1 site occurring across the codons for amino acid 51 and 52 of neo. This linker encoded the necessary DNA elements to create an artificial splice donor site, intervening intron and splice acceptor site within the neo gene, thus creating two separate exons, presently referred to as neo exon 1 and 2. Neo exon 1 encodes the first 51 amino acids of neo, while exon 2 encodes the remaining 203 amino acids plus the stop codon of the protein. A Not1 cloning site was also created within the intron.

Neo exon 2 was further subdivided to produce neo exons 2 and 3. This was achieved as follows: A set of PCR primers were designed to amplify a region of DNA encoding neo exon 1, intron and the first 111 2/3 amino acids of exon2. The 3' PCR primer resulted in the introduction of a new 5' splice site immediately after the second nucleotide of the codon for amino acid 111 in exon 2, therefore generating a new smaller exon 2. The DNA fragment now encoding the original exon 1, intron and new exon 2 was then subcloned and propagated in a pBR based vector. The remainder of the original exon 2 was used as a template for another round of PCR amplification, which generated "exon3". The 5' primer for this round of amplification introduced a new splice acceptor site at the 5' side of the newly created exon 3, i.e. before the final nucleotide of the codon for amino acid 111. The resultant 3 exons of neo encode the

- 29 -

following information: exon 1 - the first 51 amino acids of neo; exon 2 - the next 111 2/3 amino acids, and exon 3 the final 91 1/3 amino acids plus the translational stop codon of the neo gene.

5           Neo exon 3 was incorporated along with the above mentioned DNA elements into the marking plasmid "Desmond". Neo exons 1 and 2 were incorporated into the targeting plasmid "Molly". The NotI cloning site created within the intron between exons 1 and 2 was used in  
10 subsequent cloning steps to insert genes of interest into the targeting plasmid.

          A second targeting plasmid "Mandy" was also generated. This plasmid is almost identical to "Molly" (some restriction sites on the vector have been changed)  
15 except that the original HisD and DHFR genes contained in "Molly" were inactivated. These changes were incorporated because the Desmond cell line was no longer being cultured in the presence of Histidinol, therefore it seemed unnecessary to include a second copy of the  
20 HisD gene. Additionally, the DHFR gene was inactivated to ensure that only a single DHFR gene, namely the one present in the Desmond marked site, would be amplifiable in any resulting cell lines. "Mandy" was derived from "Molly" by the following modifications:

25           (i) A synthetic linker was inserted in the middle of the DHFR coding region. This linker created a stop codon and shifted the remainder of the DHFR coding

- 30 -

region out of frame, therefore rendering the gene nonfunctional.

(ii) A portion of the HisD gene was deleted and replaced with a PCR generated HisD fragment lacking the promoter and start codon of the gene.

Figure 1 depicts the arrangement of these DNA elements in the marker plasmid "Desmond". Figure 2 depicts the arrangement of these elements in the first targeting plasmid, "Molly". Figure 3 illustrates the possible arrangement in the CHO genome, of the various DNA elements after targeting and integration of Molly DNA into Desmond marked CHO cells. Figure 9 depicts the targeting plasmid "Mandy."

Construction of the marking and targeting plasmids from the above listed DNA elements was carried out following conventional cloning techniques (see, e.g., Molecular Cloning, A Laboratory Manual, J. Sambrook et al, 1987, Cold Spring Harbor Laboratory Press, and Current Protocols in Molecular Biology, F. M. Ausubel et al, eds., 1987, John Wiley and Sons). All plasmids were propagated and maintained in E. coli XLI blue (Stratagene, cat. # 200236). Large scale plasmid preparations were prepared using Promega Wizard Maxiprep DNA Purification System®, according to the manufacturer's directions.

- 31 -

## EXAMPLE 2

### Construction of a Marked CHO Cell Line

#### 1. Cell Culture and Transfection Procedures to Produced Marked CHO Cell Line

5           Marker plasmid DNA was linearized by digestion overnight at 37°C with Bst1107I. Linearized vector was ethanol precipitated and resuspended in sterile TE to a concentration of 1mg/ml. Linearized vector was introduced into DHFR-Chinese hamster ovary cells (CHO cells)  
10    DG44 cells (Urlaub et al, *Som. Cell and Mol. Gen.*, 12:555-566 (1986)) by electroporation as follows.

          Exponentially growing cells were harvested by centrifugation, washed once in ice cold SBS (sucrose buffered solution, 272mM sucrose, 7mM sodium phosphate, pH 7.4, 1mM magnesium chloride) then resuspended in SBS  
15    to a concentration of  $10^7$  cells/ml. After a 15 minute incubation on ice, 0.4ml of the cell suspension was mixed with 40 $\mu$ g linearized DNA in a disposable electroporation cuvette. Cells were shocked using a BTX  
20    electrocell manipulator (San Diego, CA) set at 230 volts, 400 microfaraday capacitance, 13 ohm resistance. Shocked cells were then mixed with 20 ml of prewarmed CHO growth media (CHO-S-SFMII, Gibco/BRL, catalog # 31033-012) and plated in 96 well tissue culture plates.  
25    Forty eight hours after electroporation, plates were fed with selection media (in the case of transfection with Desmond, selection media is CHO-S-SFMII without

- 32 -

hypoxanthine or thymidine, supplemented with 2mM  
Histidinol (Sigma catalog # H6647)). Plates were main-  
tained in selection media for up to 30 days, or until  
some of the wells exhibited cell growth. These cells  
5 were then removed from the 96 well plates and expanded  
ultimately to 120 ml spinner flasks where they were  
maintained in selection media at all times.

### EXAMPLE 3

#### Characterization of Marked CHO Cell Lines

##### 10 (a) Southern Analysis

Genomic DNA was isolated from all stably growing  
Desmond marked CHO cells. DNA was isolated using the  
Invitrogen Easy® DNA kit, according to the manufactur-  
er's directions. Genomic DNA was then digested with  
15 HindIII overnight at 37°C, and subjected to Southern  
analysis using a PCR generated digoxigenin labelled  
probe specific to the DHFR gene. Hybridizations and  
washes were carried out using Boehringer Mannheim's DIG  
easy hyb (catalog # 1603 558) and DIG Wash and Block  
20 Buffer Set (catalog # 1585 762) according to the manu-  
facturer's directions. DNA samples containing a single  
band hybridizing to the DHFR probe were assumed to be  
Desmond clones arising from a single cell which had  
integrated a single copy of the plasmid. These clones  
25 were retained for further analysis. Out of a total of  
45 HisD resistant cell lines isolated, only 5 were

- 33 -

single copy integrants. Figure 4 shows a Southern blot containing all 5 of these single copy Desmond clones. Clone names are provided in the figure legend.

(b) Northern Analysis

5           Total RNA was isolated from all single copy Desmond clones using TRIzol reagent (Gibco/BRL cat # 15596-026) according to the manufacturer's directions. 10-20 $\mu$ g RNA from each clone was analyzed on duplicate formaldehyde gels. The resulting blots were probed with PCR  
10   generated digoxigenin labelled DNA probes to (i) DHFR message, (ii) HisD message and (iii) CAD message. CAD is a trifunctional protein involved in uridine biosynthesis (Wahl et al, *J. Biol. Chem.*, 254, 17:8679-8689 (1979)), and is expressed equally in all cell  
15   types. It is used here as an internal control to help quantitate RNA loading. Hybridizations and washes were carried out using the above mentioned Boehringer Mannheim reagents. The results of the Northern analysis are shown in Figure 5. The single copy Desmond clone  
20   exhibiting the highest levels of both the His D and DHFR message is clone 15C9, shown in lane 4 in both panels of the figure. This clone was designated as the "marked cell line" and used in future targeting experiments in CHO, examples of which are presented in the following  
25   sections.

- 34 -

EXAMPLE 4Expression of Anti-CD20 Antibody  
in Desmond Marked CHO Cells

C2B8, a chimeric antibody which recognizes B-cell surface antigen CD20, has been cloned and expressed previously in our laboratory. (Reff et al, *Blood*, 83:434-45 (1994)). A 4.1 kb DNA fragment comprising the C2B8 light and heavy chain genes, along with the necessary regulatory elements (eukaryotic promoter and polyadenylation signals) was inserted into the artificial intron created between exons 1 and 2 of the neo gene contained in a pBR derived cloning vector. This newly generated 5kb DNA fragment (comprising neo exon 1, C2B8 and neo exon 2) was excised and used to assemble the targeting plasmid Molly. The other DNA elements used in the construction of Molly are identical to those used to construct the marking plasmid Desmond, identified previously. A complete map of Molly is shown in Fig. 2.

The targeting vector Molly was linearized prior to transfection by digestion with *Kpn*I and *Pac*I, ethanol precipitated and resuspended in sterile TE to a concentration of 1.5mg/mL. Linearized plasmid was introduced into exponentially growing Desmond marked cells essentially as described, except that 80µg DNA was used in each electroporation. Forty eight hours postelectroporation, 96 well plates were supplemented with selection medium - CHO-SSFMII supplemented with 400 µg/mL Geneti-

- 35 -

cin (G418, Gibco/BRL catalog # 10131-019). Plates were maintained in selection medium for up to 30 days, or until cell growth occurred in some of the wells. Such growth was assumed to be the result of clonal expansion of a single G418 resistant cell. The supernatants from all G418 resistant wells were assayed for C2B8 production by standard ELISA techniques, and all productive clones were eventually expanded to 120mL spinner flasks and further analyzed.

10 Characterization of Antibody secreting Targeted Cells

A total of 50 electroporations with Molly targeting plasmid were carried out in this experiment, each of which was plated into separate 96 well plates. A total of 10 viable, anti-CD20 antibody secreting clones were obtained and expanded to 120ml spinner flasks. Genomic DNA was isolated from all clones, and Southern analyses were subsequently performed to determine whether the clones represented single homologous recombination events or whether additional random integrations had occurred in the same cells. The methods for DNA isolation and Southern hybridization were as described in the previous section. Genomic DNA was digested with EcoRI and probed with a PCR generated digoxigenin labelled probe to a segment of the CD20 heavy chain constant region. The results of this Southern analysis are presented in figure 6. As can be seen in the figure, 8 of

- 36 -

the 10 clones show a single band hybridizing to the CD20 probe, indicating a single homologous recombination event has occurred in these cells. Two of the ten, clones 24G2 and 28C9, show the presence of additional band(s), indicative of an additional random integration elsewhere in the genome.

We examined the expression levels of anti-CD20 antibody in all ten of these clones, the data for which is shown in Table 1, below.

Table 1:

Expression Level of Anti-CD20  
Secreting Homologous Integrants

<u>Clone</u>	<u>Anti-CD20, pg/c/d</u>
20F4	3.5
25E1	2.4
42F9	1.8
39G11	1.5
21C7	1.3
50G10	0.9
29F9	0.8
5F9	0.3
-----	
28C9*	4.5
24G2*	2.1

- 37 -

\* These clones contained additional randomly integrated copies of anti-CD20. Expression levels of these clones therefore reflect a contribution from both the homologous and random sites.

5

Expression levels are reported as picogram per cell per day (pg/c/d) secreted by the individual clones, and represented the mean levels obtained from three separate ELISAs on samples taken from 120 mL spinner flasks.

10

As can be seen from the data, there is a variation in antibody secretion of approximately ten fold between the highest and lowest clones. This was somewhat unexpected as we anticipated similar expression levels from all clones due to the fact the anti-CD20 genes are all integrated into the same Desmond marked site. Nevertheless, this observed range in expression extremely small in comparison to that seen using any traditional random integration method or with our translationally impaired vector system.

15

20

Clone 20F4, the highest producing single copy integrant was selected for further study. Table 2 (below) presents ELISA and cell culture data from seven day production runs of this clone.

- 38 -

Table 2:

## 7 Day Production Run Data for 20F4

Day	% Viable	Viable/ml (x 10 <sup>5</sup> )	Tx2 (hr)	mg/L	pg/c/d
1	96	3.4	31	1.3	4.9
2	94	6	29	2.5	3.4
3	94	9.9	33	4.7	3.2
4	90	17.4	30	6.8	3
5	73	14		8.3	
6	17	3.5		9.5	

10 Clone 20F4 was seeded at  $2 \times 10^5$  ml in a 120ml spinner flask on day 0. On the following six days, cell counts were taken, doubling times calculated and 1ml samples of supernatant removed from the flask and analyzed for secreted anti-CD20 by ELISA.

15 This clone is secreting on average, 3-5pg antibody/-cell/day, based on this ELISA data. This is the same level as obtained from other high expressing single copy clones obtained previously in our laboratory using the previously developed translationally impaired random  
20 integration vectors. This result indicates the following:

(1) that the site in the CHO genome marked by the Desmond marking vector is highly transcriptionally active, and therefore represents an excellent site from  
25 which to express recombinant proteins, and

- 39 -

(2) that targeting by means of homologous recombination can be accomplished using the subject vectors and occurs at a frequency high enough to make this system a viable and desirable alternative to random integration methods.

To further demonstrate the efficacy of this system, we have also demonstrated that this site is amplifiable, resulting in even higher levels of gene expression and protein secretion. Amplification was achieved by plating serial dilutions of 20F4 cells, starting at a density of  $2.5 \times 10^4$  cells/ml, in 96 well tissue culture dishes, and culturing these cells in media (CHO-SSFMII) supplemented with 5, 10, 15 or 20nM methotrexate. Antibody secreting clones were screened using standard ELISA techniques, and the highest producing clones were expanded and further analyzed. A summary of this amplification experiment is presented in Table 3 below.

- 40 -

Table 3:

## Summary of 20F4 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
10	56	3-13	4	10-15
15	27	2-14	3	15-18
20	17	4-11	1	ND

Methotrexate amplification of 20F4 was set up as described in the text, using the concentrations of methotrexate indicated in the above table. Supernatants from all surviving 96 well colonies were assayed by ELISA, and the range of anti-CD20 expressed by these clones is indicated in column 3. Based on these results, the highest producing clones were expanded to 120ml spinners and several ELISAs conducted on the spinner supernatants to determine the pg/cell/day expression levels, reported in column 5.

The data here clearly demonstrates that this site can be amplified in the presence of methotrexate. Clones from the 10 and 15nM amplifications were found to produce on the order of 15-20pg/cell/day.

A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell.

A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated

- 41 -

from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell. The clone was then subjected to a further round of methotrexate amplification. As described above, serial  
5 dilutions of the culture were plated into 96 well dishes and cultured in CHO-SS-FMII medium supplemented with 200, 300 or 400nM methotrexate. Surviving clones were screened by ELISA, and several high producing clones were expanded to spinner cultures and further analyzed.  
10 A summary of this second amplification experiment is presented in Table 4.

Table 4:

## Summary of 20F4-15A5 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d, spinner
15 200	67	23-70	1	50-60
250	86	21-70	4	55-60
300	81	15-75	3	40-50

20 Methotrexate amplifications of 20F4-15A5 were set up and assayed as described in the text. The highest producing wells, the numbers of which are indicated in column 4, were expanded to 120ml spinner flasks. The expression levels of the cell lines derived from these wells is recorded as pg/c/d in column 5.

The highest producing clone came from the 250nM methotrexate amplification. The 250nM clone, 20F4-15A5-250A6  
25 originated from a 96 well plate in which only wells

- 42 -

grew, and therefore is assumed to have arisen from a single cell. Taken together, the data in Tables 3 and 4 strongly indicates that two rounds of methotrexate amplification are sufficient to reach expression levels of 5 60pg/cell/day, which is approaching the maximum secretion capacity of immunoglobulin in mammalian cells (Reff, M.E., *Curr. Opin. Biotech.*, 4:573-576 (1993)). The ability to reach this secretion capacity with just two amplification steps further enhances the utility of 10 this homologous recombination system. Typically, random integration methods require more than two amplification steps to reach this expression level and are generally less reliable in terms of the ease of amplification. Thus, the homologous system offers a more efficient and 15 time saving method of achieving high level gene expression in mammalian cells.

#### EXAMPLE 5

##### Expression of Anti-Human CD23 Antibody in Desmond Marked CHO Cells

20 CD23 is low affinity IgE receptor which mediates binding of IgE to B and T lymphocytes (Sutton, B.J., and Gould, H.J., *Nature*, 366:421-428 (1993)). Anti-human CD23 monoclonal antibody 5E8 is a human gamma-1 monoclonal antibody recently cloned and expressed in our 25 laboratory. This antibody is disclosed in commonly

- 43 -

assigned Serial No. 08/803,085, filed on February 20, 1997.

5 The heavy and light chain genes of 5E8 were cloned into the mammalian expression vector N5KG1, a derivative of the vector NEOSPLA (Barnett et al, in *Antibody Expression and Engineering*, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)) and two modifications were then made to the genes. We have recently observed somewhat higher secretion of immunoglobulin light chains compared to heavy chains in other expression constructs in the laboratory (Reff et al, 1997, unpublished observations). In an attempt to compensate for this deficit, we altered the 5E8 heavy chain gene by the addition of a stronger promoter/enhancer element immediately upstream of the start site. In subsequent steps, a 2.9kb DNA fragment comprising the 5E8 modified light and heavy chain genes was isolated from the N5KG1 vector and inserted into the targeting vector Mandy. Preparation of 5E8-containing Molly and electroporation into Desmond 15C9 CHO cells was essentially as described in the preceding section.

20 One modification to the previously described protocol was in the type of culture medium used. Desmond marked CHO cells were cultured in protein-free CD-CHO medium (Gibco-BRL, catalog # AS21206) supplemented with 3mg/L recombinant insulin (3mg/mL stock, Gibco-BRL, catalog # AS22057) and 8mM L-glutamine (200mM stock, Gibco-BRL, catalog # 25030-081). Subsequently, trans-

- 44 -

fectected cells were selected in the above medium supplemented with 400 $\mu$ g/mL geneticin. In this experiment, 20 electroporations were performed and plated into 96 well tissue culture dishes. Cells grew and secreted anti-CD23 in a total of 68 wells, all of which were assumed to be clones originating from a single G418 cell. Twelve of these wells were expanded to 120ml spinner flasks for further analysis. We believe the increased number of clones isolated in this experiment (68 compared with 10 for anti-CD20 as described in Example 4) is due to a higher cloning efficiency and survival rate of cells grown in CD-CHO medium compared with CHO-SS-FMII medium. Expression levels for those clones analyzed in spinner culture ranged from 0.5-3pg/c/d, in close agreement with the levels seen for the anti-CD20 clones. The highest producing anti-CD23 clone, designated 4H12, was subjected to methotrexate amplification in order to increase its expression levels. This amplification was set up in a manner similar to that described for the anti-CD20 clone in Example 4. Serial dilutions of exponentially growing 4H12 cells were plated into 96 well tissue culture dishes and grown in CD-CHO medium supplemented with 3mg/L insulin, 8mM glutamine and 30, 35 or 40nM methotrexate. A summary of this amplification experiment is presented in Table 5.

Table 5:

- 45 -

## Summary of 2H12 Amplification

nM MTX	# Wells Assayed	Expression Level mg/1 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
30	100	6-24	8	10-25
35	64	4-27	2	10-15
40	96	4-20	1	ND

The highest expressing clone obtained was a 30nM clone, isolated from a plate on which 22 wells had grown. This clone, designated 4H12-30G5, was reproducibly secreting 18-22pg antibody per cell per day. This is the same range of expression seen for the first amplification of the anti CD20 clone 20F4 (clone 20F4-15A5 which produced 15-18pg/c/d, as described in Example 4). This data serves to further support the observation that amplification at this marked site in CHO is reproducible and efficient. A second amplification of this 30nM cell line is currently underway. It is anticipated that saturation levels of expression will be achievable for the anti-CD23 antibody in just two amplification steps, as was the case for anti-CD20.

EXAMPLE 6Expression of Immunoadhesin in Desmond Marked CHO Cells

CTLA-4, a member of the Ig superfamily, is found on the surface of T lymphocytes and is thought to play a role in antigen-specific T-cell activation (Dariavach et al, *Eur. J. Immunol.*, 18:1901-1905 (1988); and Linsley et al, *J. Exp. Med.*, 174:561-569 (1991)). In order to further study the precise role of the CTLA-4 molecule in the activation pathway, a soluble fusion protein comprising the extracellular domain of CTLA-4 linked to a truncated form of the human IgG1 constant region was

- 46 -

created (Linsley et al (Id.)). We have recently expressed this CTLA-4 Ig fusion protein in the mammalian expression vector BLECH1, a derivative of the plasmid NEOSPLA (Barnett et al, in Antibody Expression and Engineering, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)).  
5 An 800bp fragment encoding the CTLA-4 Ig was isolated from this vector and inserted between the SacII and BglII sites in Molly.

Preparation of CTLA-4Ig-Molly and electroporation  
10 into Desmond clone 15C9 CHO cells was performed as described in the previous example relating to anti-CD20. Twenty electroporations were carried out, and plated into 96 well culture dishes as described previously. Eighteen CTLA-4 expressing wells were isolated from the  
15 96 well plates and carried forward to the 120ml spinner stage. Southern analyses on genomic DNA isolated from each of these clones were then carried out to determine how many of the homologous clones contained additional random integrants. Genomic DNA was digested with BglII  
20 and probed with a PCR generated digoxigenin labelled probe to the human IgG1 constant region. The results of this analysis indicated that 85% of the CTLA-4 clones are homologous integrants only; the remaining 15% contained one additional random integrant. This result  
25 corroborates the findings from the expression of anti-CD20 discussed above, where 80% of the clones were single homologous integrants. Therefore, we can conclude

- 47 -

that this expression system reproducibly yields single targeted homologous integrants in at least 80% of all clones produced.

Expression levels for the homologous CTLA4-Ig clones ranged from 8-12pg/cell/day. This is somewhat higher than the range reported for anti-CD20 antibody and anti-CD23 antibody clones discussed above. However, we have previously observed that expression of this molecule using the intronic insertion vector system also resulted in significantly higher expression levels than are obtained for immunoglobulins. We are currently unable to provide an explanation for this observation.

#### EXAMPLE 7

##### Targeting Anti-CD20 to an alternate Desmond Marked CHO Cell Line

As we described in a preceding section, we obtained 5 single copy Desmond marked CHO cell lines (see Figures 4 and 5). In order to demonstrate that the success of our targeting strategy is not due to some unique property of Desmond clone 15C9 and limited only to this clone, we introduced anti-CD20 Molly into Desmond clone 9B2 (lane 6 in figure 4, lane 1 in figure 5). Preparation of Molly DNA and electroporation into Desmond 9B2 was exactly as described in the previous example pertaining to anti-CD20. We obtained one homologous integrant from this experiment. This clone was expanded to a 120ml

- 48 -

spinner flask, where it produced on average 1.2pg anti-CD20/cell/day. This is considerably lower expression than we observed with Molly targeted into Desmond 15C9. However, this was the anticipated result, based on our  
5 northern analysis of the Desmond clones. As can be seen in Figure 5, mRNA levels from clone 9B2 are considerably lower than those from 15C9, indicating the site in this clone is not as transcriptionally active as that in  
15C9. Therefore, this experiment not only demonstrates  
10 the reproducibility of the system - presumably any marked Desmond site can be targeted with Molly - it also confirms the northern data that the site in Desmond 15C9 is the most transcriptionally active.

From the foregoing, it will be appreciated that,  
15 although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without diverting from the scope of the invention. Accordingly, the invention is not limited by the appended claims.

- 49 -

WHAT IS CLAIMED IS:

1. A method for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises the following steps:

5 (i) transfecting or transforming a mammalian cell with a first plasmid ("marker plasmid") containing the following sequences:

(a) a region of DNA that is heterologous to the mammalian cell genome which when integrated in the mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first selectable marker protein; and

15 (c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid;

(ii) selecting a cell which contain the marker plasmid integrated in its genome;

20 (iii) transfecting or transforming said selected cell with a second plasmid ("target plasmid") which contains the following sequences:

(a) a region of DNA that is identical or is sufficiently homologous to the unique region in the marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

- 50 -

(b) a DNA fragment encoding a portion of the same selectable marker contained in the marker plasmid, wherein the active selectable marker protein encoded by said DNA is only produced if said fragment is expressed  
5 in association with the fragment of said selectable marker DNA contained in the marker plasmid; and

(iv) selecting cells which contain the target plasmid integrated at the target site by screening for the expression of the first selectable marker protein.

10 2. The method of Claim 1, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

3. The method of Claim 2, wherein the second plasmid contains the remaining exons of said first  
15 selectable marker.

4. The method of Claim 3, wherein at least one DNA encoding a desired protein is inserted between said exons of said first selectable marker contained in the target plasmid.

20 5. The method Claim 4, wherein a DNA encoding a dominant selectable marker is further inserted between the exons of said first selectable marker contained in

- 51 -

the target plasmid to provide for co-amplification of the DNA encoding the desired protein.

6. The method of Claim 3, wherein the first dominant selectable marker is selected from the group consisting of neomycin phosphotransferase, histidinol dehydrogenase, dihydrofolate reductase, hygromycin phosphotransferase, herpes simplex virus thymidine kinase, adenosine deaminase, glutamine synthetase, and hypoxanthine-guanine phosphoribosyl transferase.

7. The method of Claim 4, wherein the desired protein is a mammalian protein.

8. The method of Claim 7, wherein the protein is an immunoglobulin.

9. The method of Claim 1, which further comprises determining the RNA levels of the selectable marker (c) contained in the marker plasmid prior to integration of the target vector.

10. The method of Claim 9, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex

- 52 -

thymidine kinase, hydromycin phosphotransferase, adenosine deaminase and glutamine synthetase.

11. The method of Claim 1, wherein the mammalian cell is selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

12. The method of Claim 11, wherein the cell is a CHO cell.

13. The method of Claim 1, wherein the marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains the first two exons of the neomycin phosphotransferase gene.

14. The method of Claim 1, wherein the marker plasmid further contains a rare restriction endonuclease sequence which is inserted within the region of homology.

15. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic DNA.

- 53 -

16. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is at least 300 nucleotides.

17. The method of Claim 16, wherein the unique  
5 region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

18. The method of claim 17, wherein the unique region of DNA preferably ranges in size from 2 to 10 kilobases.

10 19. The method of Claim 1, wherein the first selectable marker DNA is split into at least three exons.

20. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination  
15 is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

21. The method of Claim 20, wherein the unique region of DNA does not contain any functional genes.

22. A vector system for inserting a desired DNA at  
20 a target site in the genome of a mammalian cell which comprises at least the following:

- 54 -

(i) a first plasmid ("marker plasmid") containing at least the following sequences:

5 (a) a region of DNA that is heterologous to the mammalian cell genome which when integrated in the mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first selectable marker protein; and

10 (c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid; and

(ii) a second plasmid ("target plasmid") which contains at least the following sequences:

15 (a) a region of DNA that is identical or is sufficiently homologous to the unique region in the marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

20 (b) a DNA fragment encoding a portion of the same selectable marker contained in the marker plasmid, wherein the active selectable marker protein encoded by said DNA is only produced if said fragment is expressed in association with the fragment of said selectable marker DNA contained in the marker plasmid.

- 55 -

23. The vector system of Claim 22, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

24. The vector system of Claim 23, wherein the  
5 second plasmid contains the remaining exons of said first selectable marker.

25. The vector system of Claim 24, wherein at least one DNA encoding a desired protein is inserted between said exons of said first selectable marker con-  
10 tained in the target plasmid.

26. The vector system of Claim 24, wherein a DNA encoding a dominant selectable marker is further inserted between the exons of said first selectable marker contained in the target plasmid to provide for co-ampli-  
15 fication of the DNA encoding the desired protein.

27. The vector system of Claim 24, wherein the first dominant selectable marker is selected from the group consisting of neomycin phosphotransferase, histidinol dehydrogenase, dihydrofolate reductase,  
20 hygromycin phosphotransferase, herpes simplex virus thymidine kinase, adenosine deaminase, glutamine synthetase, and hypoxanthine-guanine phosphoribosyl transferase.

- 56 -

28. The vector system of Claim 25, wherein the desired protein is a mammalian protein.

29. The vector system of Claim 28, wherein the protein is an immunoglobulin.

5        30. The vector system of Claim 22, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex thymidine kinase, hydromycin phosphotransferase, adenosine deaminase and glutamine synthetase.  
10

31. The vector system of Claim 22, which provides for insertion of a desired DNA at a targeted site in the genome of a mammalian cell selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma  
15 cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

32. The vector system of Claim 31, wherein the mammalian cell is a CHO cell.

33. The vector system of Claim 22, wherein the  
20 marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains

- 57 -

the first two exons of the neomycin phosphotransferase gene.

34. The vector system of Claim 22, wherein the marker plasmid further contains a rare restriction endo-  
5 nuclease sequence which is inserted within the region of homology.

35. The vector system of Claim 22, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic  
10 DNA.

36. The vector system of Claim 22, wherein the unique region of DNA (a) contained in the marker plasmid vector system that provides for homologous recombination is at least 300 nucleotides.

15 37. The vector system of Claim 36, wherein the unique region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

38. The vector system of Claim 37, wherein the unique region of DNA preferably ranges in size from 2 to  
20 10 kilobases.

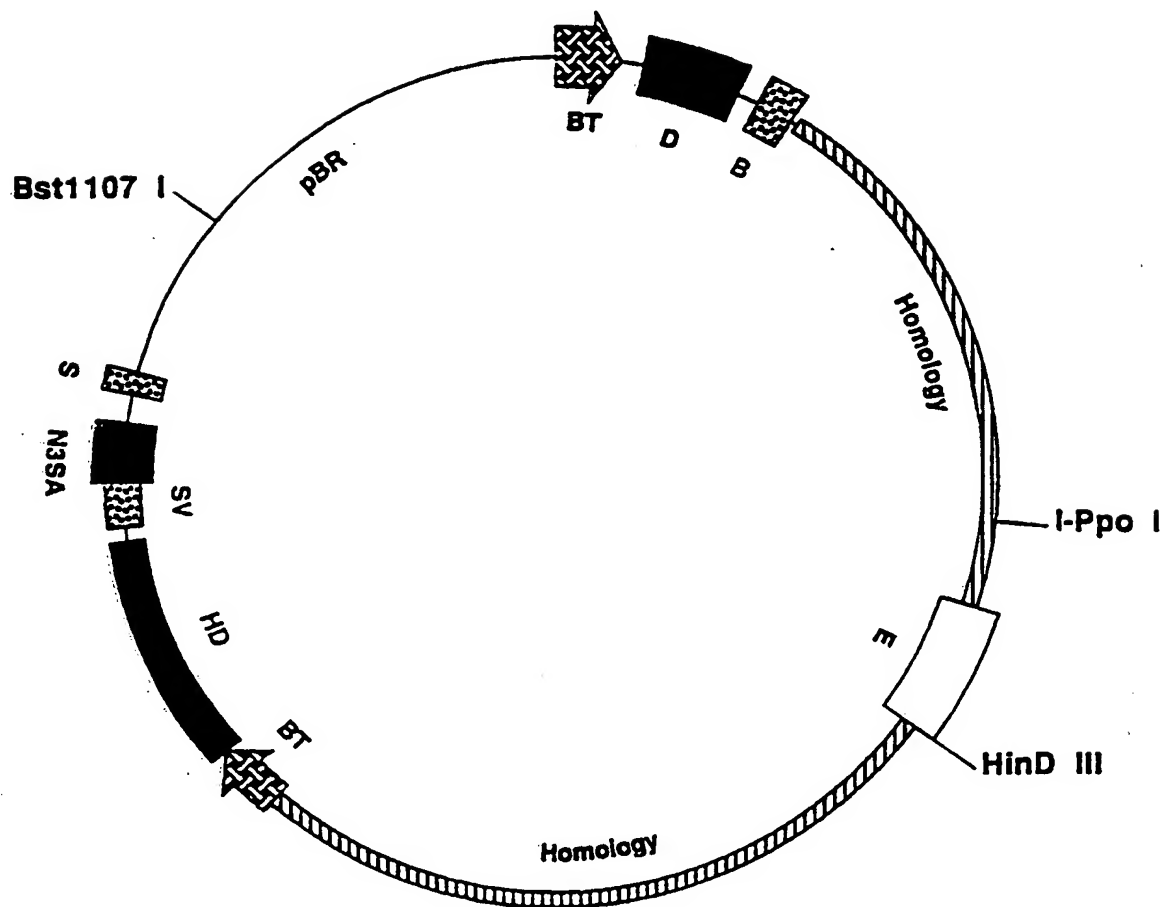
- 58 -

39. The vector system of Claim 22, wherein the first selectable marker DNA is split into at least three exons.

40. The vector system of Claim 22, wherein the  
5 unique region of DNA that provides for homologous recombination is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

41. The vector system of Claim 40, wherein the  
10 unique region of DNA does not contain any functional genes.

# DESMOND



- HD = Salmonella HisD Gene  
N3 = Neomycin Phosphotransferase Exon 3  
D = Murine Dihydrofolate reductase  
E = Cytomegalovirus and SV40 Enhancers  
SA = Splice acceptor  
BT = Mouse Beta Globin Major Promoter  
B = Bovine Growth Hormone Polyadenylation  
S = SV40 Early Polyadenylation  
SV = SV40 Late Polyadenylation

FIGURE 1A

# Desmond

14,683 bp Bst1107 I linear

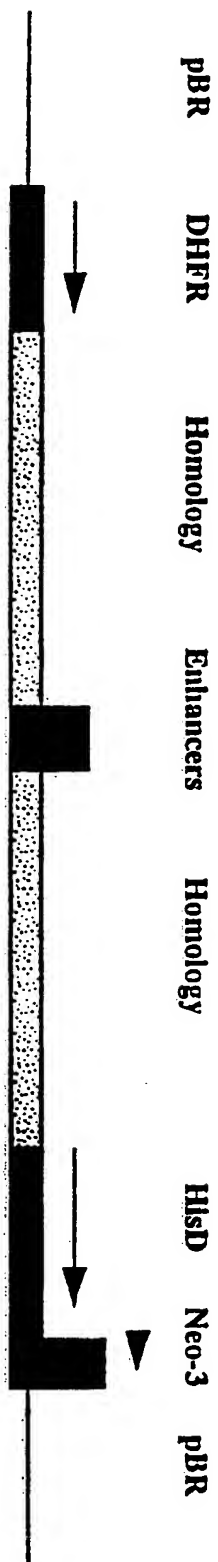
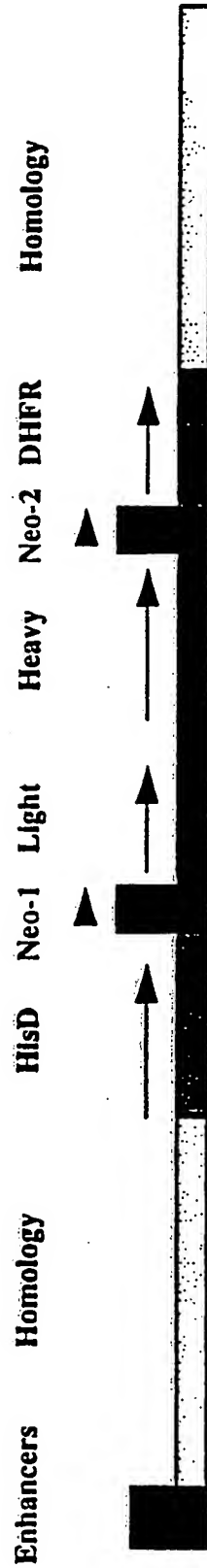


FIGURE 1B

**FIGURE 2A**

# Molly

## 15,987 bp Pac I, Kpn I fragment

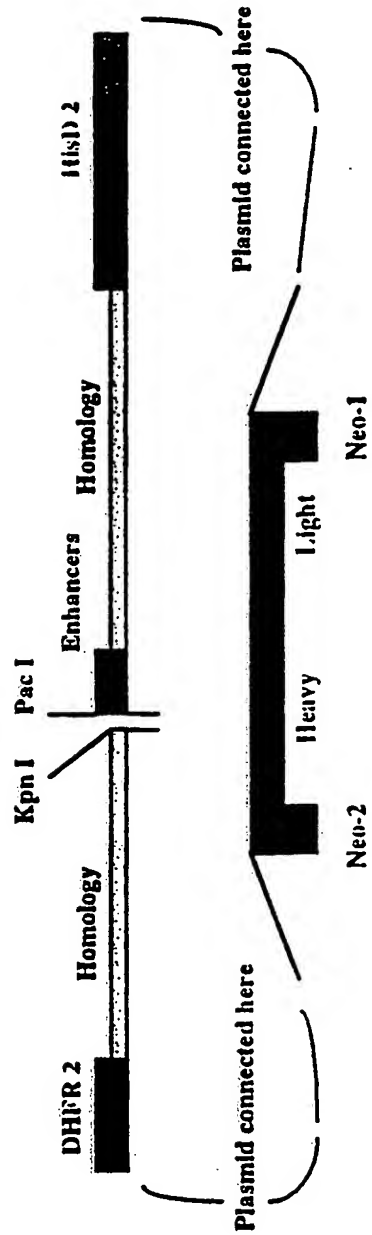


# Homologous Recombination

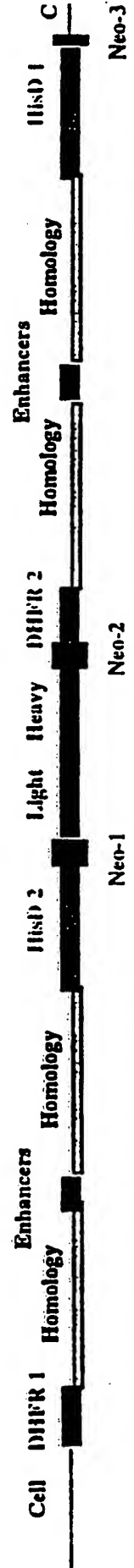
## Desmond in CHO



## Molly



## Single crossover in CHO



## Southern Analysis of Desmond Marked CHO Cells

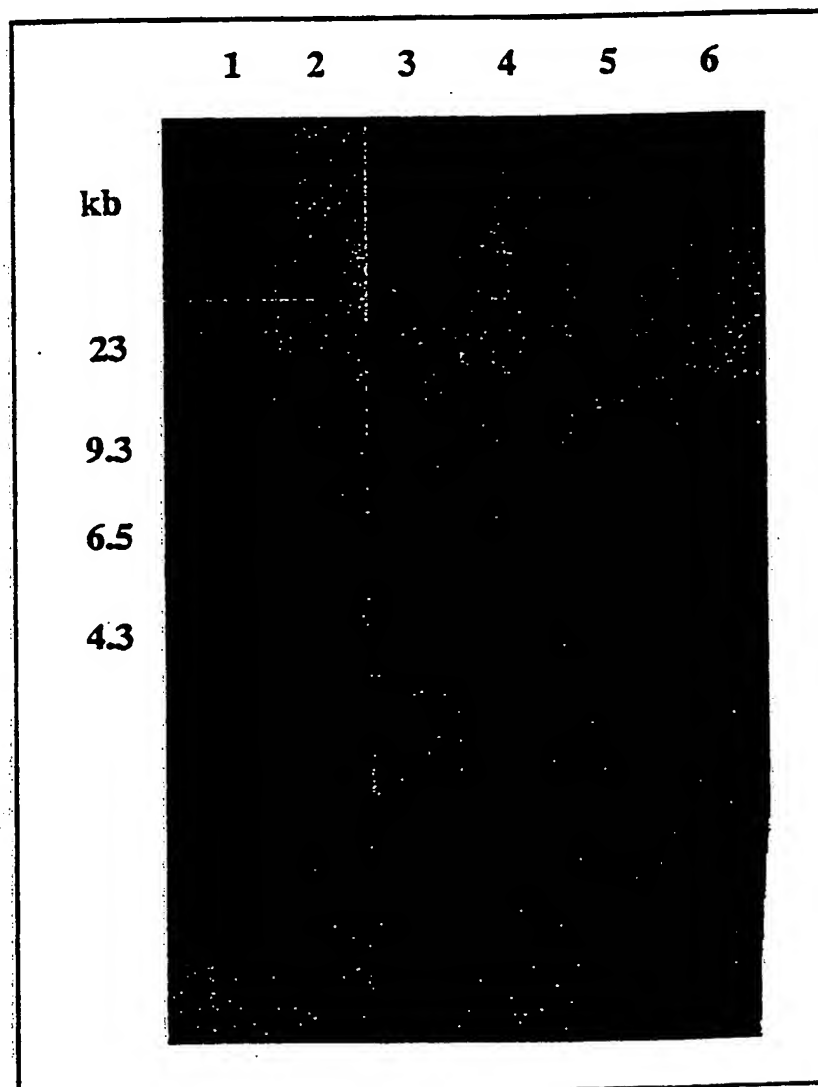


FIGURE 4

Northern Analysis of Desmond  
Marked CHO Cells

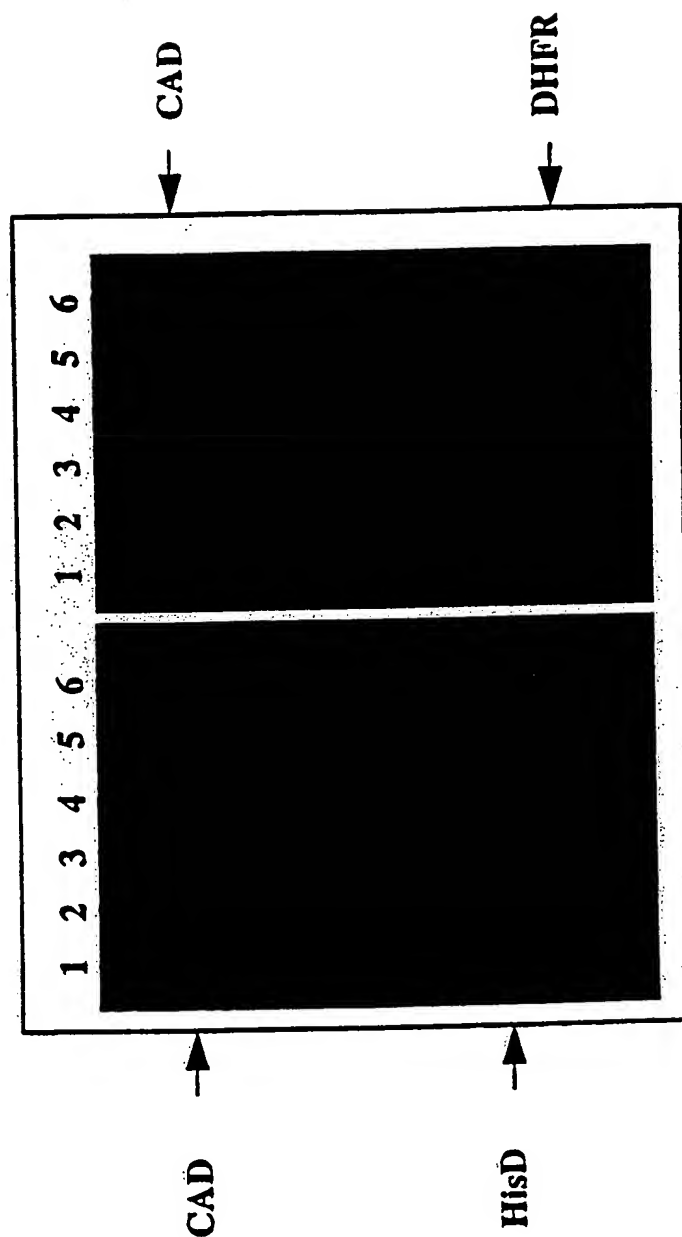


FIGURE 5

**Southern Analysis of Anti CD20**  
**Integrants in Marked CHO Cells**

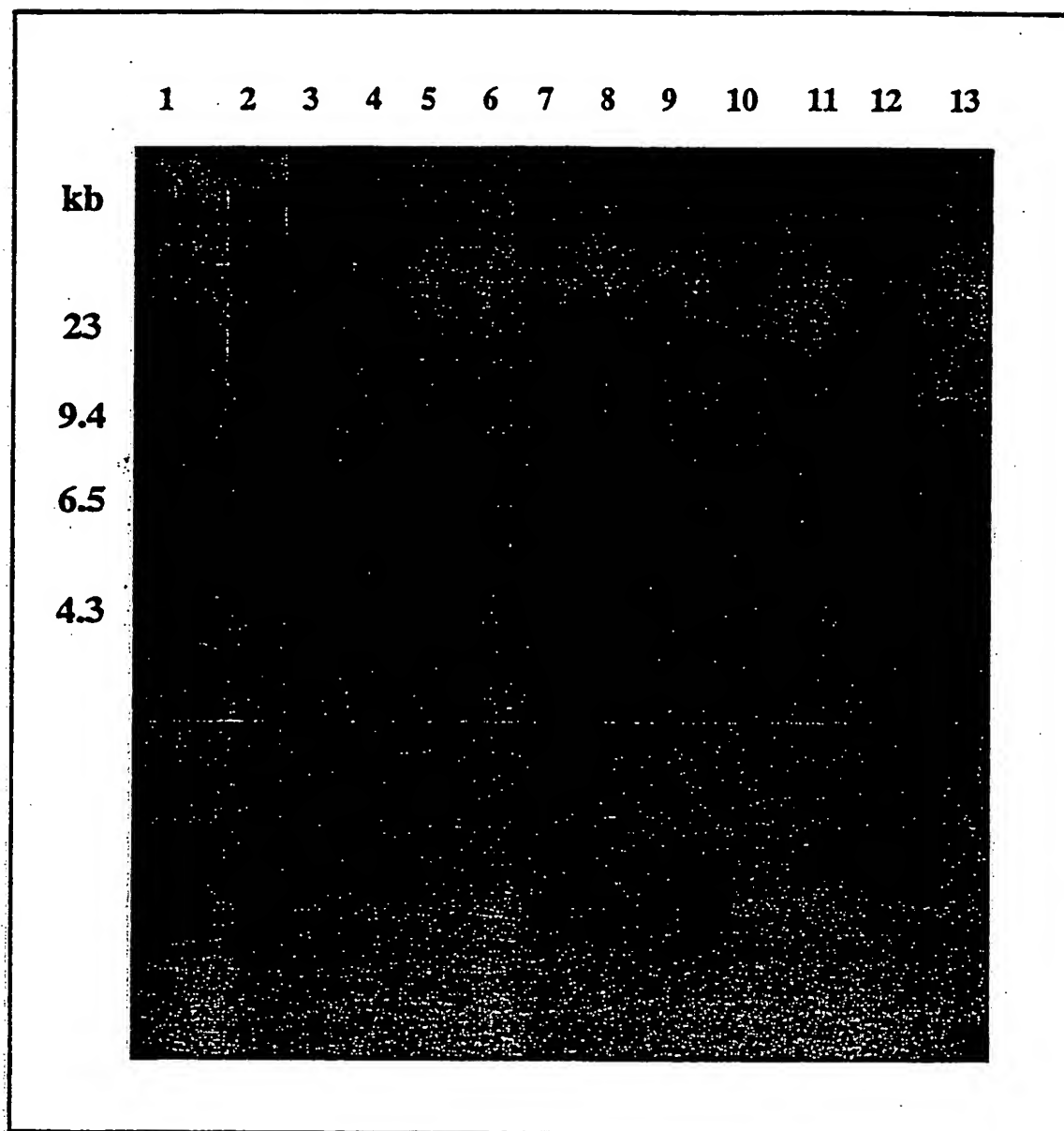


FIGURE 6

DNASIS  
Desmond

```

      10      20      30      40      50      60
TTTCTAGACC TAGGGCGGCC AGCTAGTAGC TTTGCTTCTC AATTTCTTAT TTGCATAATG

      70      80      90     100     110     120
AGAAAAAAG GAAAATTAAT TTAAACACCA ATTCAGTAGT TGATTGAGCA AATGCGTTGC

      130     140     150     160     170     180
CAAAAAGGAT GCTTTAGAGA CAGTGTCTC TGCACAGATA AGGACAAACA TTATTCAGAG

      190     200     210     220     230     240
GGAGTACCCA GAGCTGAGAC TCCTAAGCCA GTGAGTGGCA CAGCATCCAG GGAGAAATAT

      250     260     270     280     290     300
GCTTGTCTAT ACCGAAGCCT GATTCCGTAG AGCCACACCC TGGTAAGGGC CAATCTGTCT

      310     320     330     340     350     360
ACACAGGATA GAGAGGGCAG GAGCCAGGGC AGAGCATATA AGGTGAGGTA GGATCAGTTG

      370     380     390     400     410     420
CTCACAT TTGCTTCTGA CATAGTTGTG TTGGGAGCTT GGATAGCTTG GGGGGGGGAC

      430     440     450     460     470     480
AGCTCAGGGC TGCATTTCG CGCCAACTT GACGGCAATC CTAGCGTGAA GGCTGGTAGG

      490     500     510     520     530     540
ATTTTATCCC CGCTGCCATC ATGGTTCGAC CATTGAACTG CATCGTCGCC GTGTCCCAA

      550     560     570     580     590     600
ATATGGGGAT TGGCAAGAAC GGAGACCTAC CCTGGCCTCC GCTCAGGAAC GAGTTCAAGT

      610     620     630     640     650     660
ACTTCCAAAG AATGACCACA ACCTCTTCAG TGGAAGGTAA ACAGAATCTG GTGATTATGG

      670     680     690     700     710     720
GTAGGAAAAC CTGGTTCTCC ATTCCTGAGA AGAATCGACC TTAAAGGAC AGAATTAATA

      730     740     750     760     770     780
TTCTCAG TAGAGAACTC AAAGAACCAC CACGAGGAGC TCATTTTCTT GCCAAAAGTT

      790     800     810     820     830     840
TGGATGATGC CTTAAGACTT ATTGAACAAC CGGAATTGGC AAGTAAAGTA GACATGGTTT

      850     860     870     880     890     900
GGATAGTCGG AGGCAGTTCT GTTTACCAGG AAGCCATGAA TCAACCAGGC CACCTCAGAC

      910     920     930     940     950     960
TCTTTGTGAC AAGGATCATG CAGGAATTTG AAAGTGACAC GTTTTCCCA GAAATTGATT

      970     980     990    1000    1010    1020
TGGGGAAATA TAACTTCTC CCAGAATACC CAGGCGTCCT CTCTGAGGTC CAGGAGGAAA

      1030    1040    1050    1060    1070    1080
AAGGCATCAA GTATAAGTTT GAAGTCTACG AGAAGAAAGA CTAACAGGAA GATGCTTTCA

      1090    1100    1110    1120    1130    1140
AGTTCTCTGC TCCCCTCCTA AAGCTATGCA TTTTATAAG ACCATGGGAC TTTTGCTGGC

      1150    1160    1170    1180    1190    1200
TTTAGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC

      1210    1220    1230    1240    1250    1260
GTGCCTTCTT TGACCCTGGA AGGTGCCACT CCCACTGTCC TTTCTAATA AAATGAGGAA

      1270    1280    1290    1300    1310    1320
ATTGCATCGC ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC

```

FIGURE 7

DNASIS  
Desmond

1330 1340 1350 1360 1370 1380  
 AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG  
 1390 1400 1410 1420 1430 1440  
 GCTTCTGAGG CGGAAAGAAC CAGCTGGGGC TCGAAGCGGC CGCCATTTC GCTGGTGGTC  
 1450 1460 1470 1480 1490 1500  
 AGATGCGGGA TGGCGTGGGA CGCGGCGGGG AGCGTCACAC TGAGGTTTTT CGCCAGACGC  
 1510 1520 1530 1540 1550 1560  
 CACTGCTGCC AGGCGCTGAT GTGCCCCGCT TCTGACCATG CGGTCGCGTT CGGTTGCACT  
 1570 1580 1590 1600 1610 1620  
 ACGCGTACTG TGAGCCAGAG TTGCCCCGGC CTCTCCGGCT GCGGTAGTTC AGGCAGTTCA  
 1630 1640 1650 1660 1670 1680  
 ATCAACTGTT TACCTTGTGG AGCGACATCC AGAGGCACTT CACCGCTTGC CAGCGGCTTA  
 1690 1700 1710 1720 1730 1740  
 ATCCAGCG CCACCATCCA GTGCAGGAGC TCGTTATCGC TATGACGGAA CAGGTATTCC  
 1750 1760 1770 1780 1790 1800  
 CTGGTCACTT CGATGGTTTG CCCGGATAAA CGGAACTGGA AAAACTGCTG CTGGTGTTTT  
 1810 1820 1830 1840 1850 1860  
 GCTTCCGTCA GCGCTGGATG CGGCGTGCGG TCGGCAAAGA CCAGACCGTT CATAACAAGC  
 1870 1880 1890 1900 1910 1920  
 TGGCGATCGT TCGGCGTATC GCCAAAATCA CCGCCGTAAG CCGACCACGG GTTGCCGTTT  
 1930 1940 1950 1960 1970 1980  
 TCATCATATT TAATCAGCGA CTGATCCACC CAGTCCCAGA CGAAGCCGCC CTGTAAACGG  
 1990 2000 2010 2020 2030 2040  
 GGATACTGAC GAAACGCCTG CCAGTATTTA GCGAAACCGC CAAGACTGTT ACCCATCGCG  
 2050 2060 2070 2080 2090 2100  
 GGGCGTATT CGCAAAGGAT CAGCGGGCGC GTCTCTCCAG GTAGCGAAAG CCATTTTTTG  
 2110 2120 2130 2140 2150 2160  
 ATGGACCATT TCGGCACAGC CGGGAAGGGC TGGTCTTCAT CCACGCGCGC GTACATCGGG  
 2170 2180 2190 2200 2210 2220  
 CAAATAATAT CCGTGCCGT GGTGTCGGCT CCGCCGCTT CATACTGCAC CGGGCGGGAA  
 2230 2240 2250 2260 2270 2280  
 GGATCGACAG ATTTGATCCA GCGATACAGC GCGTCGTGAT TAGCGCCGTG GCCTGATTCA  
 2290 2300 2310 2320 2330 2340  
 TTCCCAGCG ACCAGATGAT CACACTCGGG TGATTACGAT CGCGCTGCAC CATTGCGGTT  
 2350 2360 2370 2380 2390 2400  
 ACGCGTTTCG TCATCGCCGG TAGCCAGCGC GGATCATCGG TCAGACGATT CATTGGCACC  
 2410 2420 2430 2440 2450 2460  
 ATGCCGTGGG TTTCAATATT GGCTTCATCC ACCACATACA GGCCGTAGCG GTCGCACAGC  
 2470 2480 2490 2500 2510 2520  
 GTGTACCACA GCGGATGGTT CGGATAATGC GAACAGCGCA CGGCGTTAAA GTTGTCTGCT  
 2530 2540 2550 2560 2570 2580  
 TTCATCAGCA GGATATCCTG CACCATCGTC TGCTCATCCA TGACCTGACC ATGCAGAGGA  
 2590 2600 2610 2620 2630 2640

DNASIS  
Desmond Park

TGATGCTCGT GACGGTTAAC GCCTCGAATC AGCAACGGCT TGCCGTTTCAG CAGCAGCAGA  
 2650 2660 2670 2680 2690 2700  
 CCATTTTCAA TCCGCACCTC GCGGAAACCG ACATCGCAGG CTTCTGCTTC AATCAGCGTG  
 2710 2720 2730 2740 2750 2760  
 CCGTCGGCGG TGTGCAGTTC AACCACCGCA CGATAGAGAT TCGGGATTTC GGCCTCCAC  
 2770 2780 2790 2800 2810 2820  
 AGTTTCGGGT TTTCGACGTT CAGACGTAGT GTGACGCGAT CGGCATAACC ACCACGCTCA  
 2830 2840 2850 2860 2870 2880  
 TCGATAATTT CACCGCCGAA AGGCGCGGTG CCGCTGGCGA CCTGCGTTTC ACCCTGCCAT  
 2890 2900 2910 2920 2930 2940  
 AAAGAAACTG TTACCCGTAG GTAGTCACGC AACTCGCCGC ACATCTGAAC TTCAGCCTCC  
 2950 2960 2970 2980 2990 3000  
 AGTACAGCGC GGCTGAAATC ATCATTAAAG CGAGTGGCAA CATGGAAATC GCTGATTTGT  
 3010 3020 3030 3040 3050 3060  
 GTAGTCGGTT TATGCAGCAA CGAGACGTCA CGGAAAATGC CGCTCATCCG CCACATATCC  
 3070 3080 3090 3100 3110 3120  
 TGATCTTCCA GATAACTGCC GTCACCTCCAG CGCAGCACCA TCACCGCGAG GCGGTTTTCT  
 3130 3140 3150 3160 3170 3180  
 CCGGCGCGTA AAAATGCGCT CAGGTCAAAT TCAGACGGCA AACGACTGTC CTGGCCGTAA  
 3190 3200 3210 3220 3230 3240  
 CCGACCCAGC GCCCCTTGCA CCACAGATGA AACGCCGAGT TAACGCCATC AAAAATAATT  
 3250 3260 3270 3280 3290 3300  
 CGCGTCTGGC CTTCTGTAG CCAGCTTTCA TCAACATTAA ATGTGAGCGA GTAACAACCC  
 3310 3320 3330 3340 3350 3360  
 GTCGGATTCT CCGTGGAAC AAACGGCGGA TTGACCGTAA TGGGATAGGT CACGTTGGTG  
 3370 3380 3390 3400 3410 3420  
 TAGATGGGCG CATCGTAACC GTGCATCTGC CAGTTTGAGG GGACGACGAC AGTATCGGCC  
 3430 3440 3450 3460 3470 3480  
 TCAGGAAGAT CGCACTCCAG CCAGCTTTCC GGCACCGCTT CTGGTGCCGG AAACCAGGCA  
 3490 3500 3510 3520 3530 3540  
 AAGCGCCATT CGCCATTGAG GCTGCGCAAC TGTTGGGAAG GGCGATCGGT GCGGGCCTCT  
 3550 3560 3570 3580 3590 3600  
 TCGCTATTAC GCCAGCTGGC GAAAGGGGGA TGTGCTGCAA GGCGATTAAG TTGGGTAACG  
 3610 3620 3630 3640 3650 3660  
 CCAGGGTTTT CCCAGTCACG ACGTTGTAAA ACGACTTAAT CCGTCGAGGG GCTGCCTCGA  
 3670 3680 3690 3700 3710 3720  
 AGCAAACGAC CTTCCGTTGT GCAGCCAGCG GCGCCTGCGC CGGTGCCAC AATCGTGCGC  
 3730 3740 3750 3760 3770 3780  
 GAACAAACTA AACCAGAACA AATTATACCG GCGGCACCGC CGCCACCACC TTCTCCCGTG  
 3790 3800 3810 3820 3830 3840  
 CCTAACATTC CAGCGCCTCC ACCACCACCA CCACCATCGA TGTCTGAATT GCCGCCCCGT  
 3850 3860 3870 3880 3890 3900  
 CCACCAATGC CGACGGAACC TCAACCCGCT GCACCTTTAG ACGACAGACA ACAATTGTTG

DNASIS  
Desmond

3910 3920 3930 3940 3950 3960  
 GAAGCTATTA GAAACGAAAA AAATCGCACT CGTCTCAGAC CGGCTCTCTT AAGGTAGCTC  
 3970 3980 3990 4000 4010 4020  
 AAACCAAAAA CGGCGCCCGA AACCAGTACA ATAGTTGAGG TGCCGACTGT GTTGCCTAAA  
 4030 4040 4050 4060 4070 4080  
 GAGACATTTG AGCCTAAACC GCCGTCTGCA TCACCGCCAC CACCTCCGCC TCCGCCTCCG  
 4090 4100 4110 4120 4130 4140  
 CCGCCAGCCC CGCCTGCGCC TCCACCGATG GTAGATTTAT CATCAGCTCC ACCACCGCCC  
 4150 4160 4170 4180 4190 4200  
 CCATTAGTAG ATTTGCCGTC TGAAATGTTA CCACCGCCTG CACCATCGCT TTCTAACGTG  
 4210 4220 4230 4240 4250 4260  
 TTGTCTGAAT TAAAATCGGG CACAGTTAGA TTGAAACCCG CCCAAAAACG CCCGCAATCA  
 4270 4280 4290 4300 4310 4320  
 TATAATTTC CAAAAAGCTC AACTACAAAT TTGATCGCGG ACGTGTTAGC CGACACAATT  
 4330 4340 4350 4360 4370 4380  
 AATAGGCGTC GTGTGGCTAT GGCAAAATCG TCTTCGGAAG CAACTTCTAA CGACGAGGGT  
 4390 4400 4410 4420 4430 4440  
 TGGGACGACG ACGATAATCG GCCTAATAAA GCTAACACGC CCGATGTAA ATATGTCCAA  
 4450 4460 4470 4480 4490 4500  
 GCTACTAGTG GTACCTTAAT TAAGGGGCGG AGAATGGGCG GAACTGGGCG GAGTTAGGGG  
 4510 4520 4530 4540 4550 4560  
 CGGGATGGGC GGAGTTAGGG GCGGGACTAT GGTTGCTGAC TAATTGAGAT GCATGCTTTG  
 4570 4580 4590 4600 4610 4620  
 CATACTTCTG CCTGCTGGGG AGCCTGGGGA CTTTCCACAC CTGGTTGCTG ACTAATTGAG  
 4630 4640 4650 4660 4670 4680  
 TGCATGCTT TGCATACTTC TGCCTGCTGG GGAGCCTGGG GACTTTCCAC ACCCTAACTG  
 4690 4700 4710 4720 4730 4740  
 ACACACATTC CACAGAATTA ATTCCCCTAG TTATTAATAG TAATCAATTA CGGGGTCATT  
 4750 4760 4770 4780 4790 4800  
 AGTTCATAGC CCATATATGG AGTTCCGCGT TACATAACTT ACGGTAAATG GCGCGCCTGG  
 4810 4820 4830 4840 4850 4860  
 CTGACCGCCC AACGACCCCC GCCCATTGAC GTCAATAATG ACGTATGTTT CCATAGTAAC  
 4870 4880 4890 4900 4910 4920  
 GCCAATAGGG ACTTTCCATT GACGTCAATG GGTGGAGTAT TTACGGTAAA CTGCCCACTT  
 4930 4940 4950 4960 4970 4980  
 GGCAGTACAT CAAGTGATC ATATGCCAAG TACGCCCCCT ATTGACGTCA ATGACGGTAA  
 4990 5000 5010 5020 5030 5040  
 ATGGCCCCGC TGGCATTATG CCCAGTACAT GACCTTATGG GACTTTCCTA CTTGGCAGTA  
 5050 5060 5070 5080 5090 5100  
 CATCTACGTA TTAGTCATCG CTATTACCAT GGTGATGCGG TTTTGGCAGT ACATCAATGG  
 5110 5120 5130 5140 5150 5160  
 GCGTGGATAG CGGTTTGACT CACGGGGATT TCCAAGTCTC CACCCCATTTG ACGTCAATGG  
 5170 5180 5190 5200 5210 5220  
 GAGTTTGTTC TGAAGCTTGG CCGGCCAGCT TTATTTAACG TGTTTACGTC GAGTCAATTG

DNASIS  
Desmond

5230 5240 5250 5260 5270 5280  
 TACACTAACG ACAGTGATGA AAGAAATACA AAAGCGCATA ATATTTTGAA CGACGTGAA  
 5290 5300 5310 5320 5330 5340  
 CCTTTATTAC AAAACAAAAC ACAAACGAAT ATCGACAAAG CTAGATTGCT GCTACAAGAT  
 5350 5360 5370 5380 5390 5400  
 TTGGCAAGTT TTGTGGCGTT GAGCGAAAAT CCATTAGATA GTCCAGCCAT CGGTTCCGAA  
 5410 5420 5430 5440 5450 5460  
 AAACAACCCT TGTTTGAAAC TAATCGAAAC CTATTTTACA AATCTATTGA GGATTTAATA  
 5470 5480 5490 5500 5510 5520  
 TTTAAATTCA GATATAAAGA CGCTGAAAAT CATTTGATTT TCGCTCTAAC ATACCACCCT  
 5530 5540 5550 5560 5570 5580  
 AAAGATTATA AATTTAATGA ATTATTAATA TACATCAGCA ACTATATATT GATAGACATT  
 5590 5600 5610 5620 5630 5640  
 CAGTTTGT GATATTAGTT TGTGCGTCTC ATTACAATGG CTGTTATTTT TAACAACAAA  
 5650 5660 5670 5680 5690 5700  
 CAACTGCTCG CAGACAATAG TATAGAAAAG GGAGGTGAAC TGTTTTGTGTT TAACGGTTCG  
 5710 5720 5730 5740 5750 5760  
 TACAACATTT TGGAAAGTTA TGTTAATCCG GTGCTGCTAA AAAATGGTGT AATTGAACTA  
 5770 5780 5790 5800 5810 5820  
 GAAGAAGCTG CGTACTATGC CGGCAACATA TTGTACAAAA CCGACGATCC CAAATTCATT  
 5830 5840 5850 5860 5870 5880  
 GATTATATAA ATTTAATAAT TAAAGCAACA CACTCCGAAG AACTACCAGA AAATAGCACT  
 5890 5900 5910 5920 5930 5940  
 GTTGTAATTT ACAGAAAAAC TATGCGCAGC GGTACTATAC ACCCCATTAA AAAAGACATA  
 5950 5960 5970 5980 5990 6000  
 ...TATTTATG ACAACAAAAA ATTTACTCTA TACGATAGAT ACATATATGG ATACGATAAT  
 6010 6020 6030 6040 6050 6060  
 AACTATGTGA ATTTTATGA GGAGAAAAAT GAAAAAGAGA AGGAATACGA AGAAGAAGAC  
 6070 6080 6090 6100 6110 6120  
 GACAAGGCGT CTAGTTTATG TGAAAAATAA ATTATATTGT CGCAAATTAA CTGTGAATCA  
 6130 6140 6150 6160 6170 6180  
 TTTGAAAATG ATTTTAAATA TTACCTCAGC GATTATAACT ACGCGTTTTC AATTATAGAT  
 6190 6200 6210 6220 6230 6240  
 AATACTACAA ATGTTCTTGT TGCCTTTGGT TTGTATCGTT AATAAAAAAC AAATTTGACA  
 6250 6260 6270 6280 6290 6300  
 TTTATAATTG TTTTATTATT CAATAATTAC AAATAGGATT GAGACCCTTG CAGTTGCCAG  
 6310 6320 6330 6340 6350 6360  
 CAAACGGACA GAGCTTGTCG AGGAGAGTTG TTGATTCATT GTTGCCTCC CTGCTGCGGT  
 6370 6380 6390 6400 6410 6420  
 TTTTCACCGA AGTTCATGCC AGTCCAGCGT TTTTGCAGCA GAAAAGCCGC CGACTTCGGT  
 6430 6440 6450 6460 6470 6480  
 TTGCGGTCGC GAGTGAAGAT CCCTTTCTTG TTACCGCAA CGCGCAATAT GCCTTGCGAG  
 6490 6500 6510 6520 6530 6540

DNASIS  
Desmond

k

GTCGCAAAT CGGCGAAATT CCATACCTGT TCACCGACGA CGGCGCTGAC GCGATCAAAG  
 6550 6560 6570 6580 6590 6600  
 ACGCGGTGAT ACATATCCAG CCATGCACAC TGATACTCTT CACTCCACAT GTCGGTGTAC  
 6610 6620 6630 6640 6650 6660  
 ATTGAGTGCA GCCC GGCTAA CGTATCCAG CCGTATTCGG TGATGATAAT CGGCTGATGC  
 6670 6680 6690 6700 6710 6720  
 AGTTTCTCCT GCCAGGCCAG AAGTTCTTTT TCCAGTACCT TCTCTGCCGT TTCAAATCG  
 6730 6740 6750 6760 6770 6780  
 CCGCTTTGGA CATACCATCC GTAATAACGG TTCAGGCACA GCACATCAA GAGATCGCTG  
 6790 6800 6810 6820 6830 6840  
 ATGGTATCGG TGTGAGCGTC GCAGAACATT ACATTGACGC AGGTGATCGG ACGCGTCGGG  
 6850 6860 6870 6880 6890 6900  
 TCGAGTTTAC GCGTTGCTTC CGCCAGTGGC GCGAAATATT CCCGTGCACC TTGCGGACGG  
 6910 6920 6930 6940 6950 6960  
 GTATCCGGTT CGTTGGCAAT ACTCCACATC ACCACGCTTG GGTGGTTTTT GTCACGCGCT  
 6970 6980 6990 7000 7010 7020  
 ATCAGCTCTT TAATCGCCTG TAAGTGCGCT TGCTGAGTTT CCCC GTTGAC TGCTCTTCG  
 7030 7040 7050 7060 7070 7080  
 CTGTACAGTT CTTTCGGCTT GTTGCCCCGT TCGAAACCAA TGCCTAAAGA GAGGTTAAAG  
 7090 7100 7110 7120 7130 7140  
 CCGACAGCAG CAGTTTCATC AATCACCAG ATGCCATGTT CATCTGCCCA GTCGAGCATC  
 7150 7160 7170 7180 7190 7200  
 TCTTCAGCGT AAGGGTAATG CGAGGTACGG TAGGAGTTGG CCCC AATCCA GTCCATTAAT  
 7210 7220 7230 7240 7250 7260  
 GCGTGGTCGT GCACCATCAG CACGTTATCG AATCCTTTGC CACGCAAGTC CGCATCTTCA  
 7270 7280 7290 7300 7310 7320  
 TGACGACCAA AGCCAGTAAA GTAGAACGGT TTGTGGTTAA TCAGGAACTG TTCGCCCTTC  
 7330 7340 7350 7360 7370 7380  
 ACTGCCACTG ACCGGATGCC GACGCGAAGC GGGTAGATAT CACACTCTGT CTGGCTTTTG  
 7390 7400 7410 7420 7430 7440  
 GCTGTGACGC ACAGTTCATA GAGATAACCT TCACCCGGTT GCCAGAGGTG CGGATTCAAC  
 7450 7460 7470 7480 7490 7500  
 ACTTGCAAAG TCCCGCTAGT GCCTTGTTCA GTTGCAACCA CCTGTTGATC CGCATCACGC  
 7510 7520 7530 7540 7550 7560  
 AGTTCAACGC TGACATCACC ATTGGCCACC ACCTGCCAGT CAACAGACGC GTGGTTACAG  
 7570 7580 7590 7600 7610 7620  
 TCTTGCGCGA CATGCGTCAC CACGGTGATA TCGTCCACCC AGGTGTTCCG CGTGGTGTAG  
 7630 7640 7650 7660 7670 7680  
 AGCATTACGC TGCGATGGAT TCCGGCATAG TTAAAGAAAT CATGGAAGTA AGACTGCTTT  
 7690 7700 7710 7720 7730 7740  
 TTCTTGCCGT TTTCGTCGGT AATCACCATT CCCGGCGGGA TAGTCTGCCA GTTCAGTTCCG  
 7750 7760 7770 7780 7790 7800  
 TTGTTACAC AAACGGTGAT ACCCCTCGAC GGATTAAAGA CTTCAAGCGG TCAACTATGA

DNASIS  
Desmond

7810 7820 7830 7840 7850 7860  
 AGAAGTGTTC GTCTTCGTCC CAGTAAGCTA TGTCTCCAGA ATGTAGCCAT CCATCCTTGT  
 7870 7880 7890 7900 7910 7920  
 CAATCAAGGC GTTGGTCGCT TCCGGATTGT TTACATAACC GGACATAATC ATAGGTCCTC  
 7930 7940 7950 7960 7970 7980  
 TGACACATAA TTCGCCTCTC TGATTAACGC CCAGCGTTTT CCCGGTATCC AGATCCACAA  
 7990 8000 8010 8020 8030 8040  
 CCTTCGCTTC AAAAAATGGA ACAACTTTAC CGACCGCGCC CGGTTTATCA TCCCCCTCGG  
 8050 8060 8070 8080 8090 8100  
 GTGTAATCAG AATAGCTGAT GTAGTCTCAG TGAGCCCATC TCCTTGTCGT ATCCCTGGAA  
 8110 8120 8130 8140 8150 8160  
 GATGGAAGCG TTTTGCAACC GCTTCCCCGA CTTCTTTTCA AAGAGGTGCG CCCCCAGAAG  
 8170 8180 8190 8200 8210 8220  
 1TTTCGTG TAAATTAGAT AAATCGTATT TGTCAATCAG AGTGCTTTTG GCGAAGAATG  
 8230 8240 8250 8260 8270 8280  
 AAAATAGGGT TGGTACTAGC AACGCACTTT GAATTTTGTA ATCCTGAAGG GATCGTAAAA  
 8290 8300 8310 8320 8330 8340  
 ACAGCTCTTC TTCAAATCTA TACATTAAGA CGACTCGAAA TCCACATATC AAATATCCGA  
 8350 8360 8370 8380 8390 8400  
 GTGTAGTAAA CATTCCAAAA CCGTGATGGA ATGGAACAAC ACTTAAAATC GCAGTATCCG  
 8410 8420 8430 8440 8450 8460  
 GAATGATTTG ATTGCCAAAA ATAGGATCTC TGGCATGCGA GAATCTGACG CAGGCAGTTC  
 8470 8480 8490 8500 8510 8520  
 TATGCGGAAG GGCCACACCC TTAGGTAACC CAGTAGATCC AGAGGAATTG TTTTGTACCG  
 8530 8540 8550 8560 8570 8580  
 CAAAGGAC TCTGGTACAA AATCGTATTC ATTAAAACCG GGAGGTAGAT GAGATGTGAC  
 8590 8600 8610 8620 8630 8640  
 GAACGTGTAC ATCGACTGAA ATCCCTGGTA ATCCGTTTTA GAATCCATGA TAATAATTTT  
 8650 8660 8670 8680 8690 8700  
 CTGGATTATT GGTAATTTTT TTTGCACGTT CAAAATTTTT TGCAACCCCT TTTTGGAAC  
 8710 8720 8730 8740 8750 8760  
 AAACACTACG GTAGGCTGCG AAATGTTTAT ACTGTTGAGC AATTCAGTT CATTATAAAT  
 8770 8780 8790 8800 8810 8820  
 GTCGTTTCGCG GGCGCAACTG CAACTCCGAT AAATAACGCG CCCAACACCG GCATAAAGAA  
 8830 8840 8850 8860 8870 8880  
 TTGAAGAGAG TTTTCACTGC ATACGACGAT TCTGTGATTT GTATTCAGCC CATATCGTTT  
 8890 8900 8910 8920 8930 8940  
 CATAGCTTCT GCCAACCGAA CGGACATTTT GAAGTATTCC GCGTACGTGA TGTTACCTC  
 8950 8960 8970 8980 8990 9000  
 GATATGTGCA TCTGTAAAAG GAATTGTTCC AGGAACCAGG GCGTATCTCT TCATAGCCTT  
 9010 9020 9030 9040 9050 9060  
 ATGCAGTTGC TCTCCAGCGG TTCCATCCTC TAGCTTTGCT TCTCAATTC TTATTTGCAT  
 9070 9080 9090 9100 9110 9120  
 AATGAGAAAA AAAGGAAAAT TAATTTTAAC ACCAATTCAG TAGTTGATTG AGCAAATGCG

DNASIS  
Desmond

```

      9130      9140      9150      9160      9170      9180
TTGCCAAAAA GGATGCTTTA GAGACAGTGT TCTCTGCACA GATAAGGACA AACATTATTTC

      9190      9200      9210      9220      9230      9240
AGAGGGAGTA CCCAGAGCTG AGACTCCTAA GCCAGTGAGT GGCACAGCAT CCAGGGAGAA

      9250      9260      9270      9280      9290      9300
ATATGCTTGT CATCACCGAA GCCTGATTCC GTAGAGCCAC ACCCTGGTAA GGGCCAATCT

      9310      9320      9330      9340      9350      9360
GCTCACACAG GATAGAGAGG GCAGGAGCCA GGGCAGAGCA TATAAGGTGA GGTAGGATCA

      9370      9380      9390      9400      9410      9420
GTTGCTCCTC ACATTTGCTT CTGACATAGT TGTGTTGGGA GCTTGGATCG ATCCACCATG

      9430      9440      9450      9460      9470      9480
GGCTTCAATA CCCTGATTGA CTGGAACAGC TGTAGCCCTG AACAGCAGCG TCGCCTGCTG

      9490      9500      9510      9520      9530      9540
A CGTCCGG CGATTTCCGC CTCTGACAGT ATTACCCGGA CGGTCAGCGA TATTTTGGAT

      9550      9560      9570      9580      9590      9600
AATGTAAAAA CGCGCGGTGA CGATGCCCTG CGTGAATACA GCGCTAAATT TGATAAAACA

      9610      9620      9630      9640      9650      9660
GAAGTGACAG CGCTACGCGT CACCCCTGAA GAGATCGCCG CCGCCGGCGC GCGTCTGAGC

      9670      9680      9690      9700      9710      9720
GACGAATTAA AACAGGCGAT GACCGCTGCC GTCAAAAATA TTGAAACGTT CCATTCCGCG

      9730      9740      9750      9760      9770      9780
CAGACGCTAC CGCCTGTAGA TGTGGAAACC CAGCCAGGCG TCGGTTGCCA GCAGGTTACG

      9790      9800      9810      9820      9830      9840
CGTCCCGTCT CGTCTGTCCG TCTGTATATT CCCGGCGGCT CGGCTCCGCT CTTCTCAACG

      9850      9860      9870      9880      9890      9900
C CTGATGC TGGCGACGCC GGCGCGCATT GCGGGATGCC AGAAGGTGGT TCTGTGCTCG

      9910      9920      9930      9940      9950      9960
CCGCCGCCCA TCGCTGATGA AATCCTCTAT GCGGCGCAAC TGTGTGGCGT GCAGGAAATC

      9970      9980      9990      10000      10010      10020
TTTAACGTCTG GCGGCGCGCA GGCGATTGCC GCTCTGGCCT TCGGCAGCGA GTCCGTACCG

      10030      10040      10050      10060      10070      10080
AAAGTGGATA AAATTTTGG CCCC GGCAAC GCCTTTGTAA CCGAAGCCAA ACGTCAGGTC

      10090      10100      10110      10120      10130      10140
AGCCAGCGTC TCGACGGCGC GGCTATCGAT ATGCCAGCCG GGCCGTCTGA AGTACTGGTG

      10150      10160      10170      10180      10190      10200
ATCGCAGACA GCGGCGCAAC ACCGGATTTC GTCGCTTCTG ACCTGCTCTC CCAGGCTGAG

      10210      10220      10230      10240      10250      10260
CACGGCCCCG ATTCCCAGGT GATCCTGCTG ACGCCTGATG CTGACATTGC CCGCAAGGTG

      10270      10280      10290      10300      10310      10320
GCGGAGGCGG TAGAACGTCA ACTGGCGGAA CTGCCGCGCG CGGACACCGC CCGGACGGCC

      10330      10340      10350      10360      10370      10380
CTGAGCGCCA GTCGTCTGAT TGTGACCAAA GATTTAGCGC AGTGCGTCGC CATCTCTAAT

      10390      10400      10410      10420      10430      10440

```

DNASIS  
Desmond rk

CAGTATGGGC CGGAACACTT AATCATCCAG ACGCGCAATG CGCGCGATTT GGTGGATGCG  
 10450 10460 10470 10480 10490 10500  
 ATTACCAGCG CAGGCTCGGT ATTTCTCGGC GACTGGTCGC CGGAATCCGC CGGTGATTAC  
 10510 10520 10530 10540 10550 10560  
 GCTTCCGGAA CCAACCATGT TTTACCGACC TATGGCTATA CTGCTACCTG TTCCAGCCTT  
 10570 10580 10590 10600 10610 10620  
 GGGTTAGCGG ATTTCCAGAA ACGGATGACC GTTCAGGAAC TGTCGAAAGC GGGCTTTTCC  
 10630 10640 10650 10660 10670 10680  
 GCTCTGGCAT CAACCATTGA AACATTGGCG GCGGCAGAAC GTCTGACCGC CCATAAAAAAT  
 10690 10700 10710 10720 10730 10740  
 GCCGTGACCC TGCGCGTAAA CGCCCTCAAG GAGCAAGCAT GAGCACTGAA AACACTCTCA  
 10750 10760 10770 10780 10790 10800  
 GCGTCGCTGA CTTAGCCCGT GAAAATGTCC GCAACCTGGA GATCCAGACA TGGATAAGAT  
 10810 10820 10830 10840 10850 10860  
 ACATTGATGA GTTTGGACAA ACCACAATA GAATGCAGTG AAAAAAATGC TTTATTTGTG  
 10870 10880 10890 10900 10910 10920  
 AAATTTGTGA TGCTATTGCT TTATTTGTAA CCATTATAAG CTGCAATAAA CAAGTTAACA  
 10930 10940 10950 10960 10970 10980  
 ACAACAATTG CATTCATTTT ATGTTTCAGG TTCAGGGGGA GGTGTGGGAG GTTTTTTAAA  
 10990 11000 11010 11020 11030 11040  
 GCAAGTAAAA CCTCTACAAA TGTGGTATGG CTGATTATGA TCTCTAGGGC CGGCCCTCGA  
 11050 11060 11070 11080 11090 11100  
 CGGCGCGCCT GGCCGCTACT AACTCTCTCC TCCCTCCTT TTCCTGCAGG CTCAAGGCGC  
 11110 11120 11130 11140 11150 11160  
 GCATGCCCCA CGGCGAGGAT CTCGTCGTGA CCCATGGCGA TGCCTGCTTG CCGAATATCA  
 11170 11180 11190 11200 11210 11220  
 TGGTGAAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG CCGGCTGGGT GTGGCGGACC  
 11230 11240 11250 11260 11270 11280  
 GCTATCAGGA CATAGCGTTG GCTACCCGTG ATATTGCTGA AGAGCTTGGC GGCGAATGGG  
 11290 11300 11310 11320 11330 11340  
 CTGACCGCTT CCTCGTGCTT TACGGTATCG CCGCTCCCGA TTCGAGCGC ATCGCCTTCT  
 11350 11360 11370 11380 11390 11400  
 ATCGCCTTCT TGACGAGTTC TTCTGAGCGG GACTCTGGGG TTCGAAATGA CCGACCAAGC  
 11410 11420 11430 11440 11450 11460  
 GACGCCCAAC CTGCCATCAC GAGATTTTGA TTCCACCGCC GCCTTCTATG AAAGGTTGGG  
 11470 11480 11490 11500 11510 11520  
 CTTCGGAATC GTTTTCCGGG ACGCCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT  
 11530 11540 11550 11560 11570 11580  
 GGAGTTCTTC GCCCAGCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA AATAAAGCAA  
 11590 11600 11610 11620 11630 11640  
 TAGCATCACA AATTTCAAA ATAAAGCATT TTTTCACTG CATTCTAGTT GTGGTTTGTG  
 11650 11660 11670 11680 11690 11700  
 CAAACTCATC AATCTATCTT ATCATGTCTG GATCGCGGCC GGTCTCTCTC TAGCCCTAGG

DNASIS  
Desmond Lark

18 / 51

11710 11720 11730 11740 11750 11760  
TCTAGACTTG GCAGAACATA TCCATCGCGT CCGCCATCTC CAGCAGCCGC ACGCCGCGCA

11770 11780 11790 11800 11810 11820  
TCTCGGGCAG CGTTGGGTCC TGGCCACGGG TGCGCATGAT CGTGCTCCTG TCGTTGAGGA

11830 11840 11850 11860 11870 11880  
CCCGGCTAGG CTGGCGGGGT TGCCTTACTG GTTAGCAGAA TGAATCACCG ATACGCGAGC

11890 11900 11910 11920 11930 11940  
GAACGTGAAG CGACTGCTGC TGCAAAACGT CTGCGACCTG AGCAACAACA TGAATGGTCT

11950 11960 11970 11980 11990 12000  
TCGGTTTCCG TGTTCGTAA AGTCTGAAA CGCGGAAGTC AGCGCCCTGC ACCATTATGT

12010 12020 12030 12040 12050 12060  
TCCGGATCTG CATCGCAGGA TGCTGCTGGC TACCCTGTGG AACACCTACA TCTGTATTAA

12070 12080 12090 12100 12110 12120  
CGAAGCGCTG GCATTGACCC TGAGTGATTT TTCTCTGGTC CCGCCGCATC CATACCGCCA

12130 12140 12150 12160 12170 12180  
GTTGTTTACC CTCACAACGT TCCAGTAACC GGGCATGTTT ATCATCAGTA ACCCGTATCG

12190 12200 12210 12220 12230 12240  
TGAGCATCCT CTCTCGTTT ATCGGTATCA TTACCCCAT GAACAGAAAT CCCCTTACA

12250 12260 12270 12280 12290 12300  
CGGAGGCATC AGTGACCAA CAGGAAAAA CCGCCCTTAA CATGGCCCGC TTTATCAGAA

12310 12320 12330 12340 12350 12360  
GCCAGACATT AACGCTTCTG GAGAACTCA ACGAGCTGGA CGCGGATGAA CAGGCAGACA

12370 12380 12390 12400 12410 12420  
TCTGTGAATC GCTTCACGAC CACGCTGATG AGCTTTACCG CAGCTGCCTC GCGCGTTTCG

12430 12440 12450 12460 12470 12480  
GTGATGACGG TGAAAACCTC TGACACATGC AGCTCCCGGA GACGGTCACA GCTTGCTCTG

12490 12500 12510 12520 12530 12540  
AAGCGGATGC CGGAGCAGA CAAGCCCGTC AGGGCGCGTC AGCGGGTGTG GCGGGGTGTC

12550 12560 12570 12580 12590 12600  
GGGGCGCAGC CATGACCCAG TCACGTAGCG ATAGCGGAGT GTATACTGGC TTAACATGTC

12610 12620 12630 12640 12650 12660  
GGCATCAGAG CAGATTGTAC TGAGAGTGCA CCATATGCGG TGTGAAATAC CGCACAGATG

12670 12680 12690 12700 12710 12720  
CGTAAGGAGA AAATACCGCA TCAGGCGCTC TTCCGCTTCC TCGCTCACTG ACTCGCTGCG

12730 12740 12750 12760 12770 12780  
CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA AAGGCGGTAA TACGGTTATC

12790 12800 12810 12820 12830 12840  
CACAGAATCA GGGGATAACG CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG

12850 12860 12870 12880 12890 12900  
GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTCCATAGG CTCCGCCCCC CTGACGAGCA

12910 12920 12930 12940 12950 12960  
TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATACCA

12970 12980 12990 13000 13010 13020  
GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCTGTT CCGACCCTGC CGCTTACCGG

DNASIS  
Desmond

13030	13040	13050	13060	13070	13080
ATACCTGTCC	GCCTTTCTCC	CTTCGGGAAG	CGTGGCGCTT	TCTCATAGCT	CACGCTGTAG
13090	13100	13110	13120	13130	13140
GTATCTCAGT	TCGGTGTAGG	TCGTTTCGCTC	CAAGCTGGGC	TGTGTGCACG	AACCCCCCT
13150	13160	13170	13180	13190	13200
TCAGCCCGAC	CGCTGCGCCT	TATCCGGTAA	CTATCGTCTT	GAGTCCAACC	CGGTAAGACA
13210	13220	13230	13240	13250	13260
CGACTTATCG	CCACTGGCAG	CAGCCACTGG	TAACAGGATT	AGCAGAGCGA	GGTATGTAGG
13270	13280	13290	13300	13310	13320
CGGTGCTACA	GAGTTCTTGA	AGTGGTGGCC	TAACACGGC	TACACTAGAA	GGACAGTATT
13330	13340	13350	13360	13370	13380
TGGTATCTGC	GCTCTGCTGA	AGCCAGTTAC	CTTCGGAAAA	AGAGTTGGTA	GCTCTTGATC
13390	13400	13410	13420	13430	13440
JCAAACAA	ACCACCGCTG	GTAGCGGTGG	TTTTTTTGT	TGCAAGCAGC	AGATTACGCG
13450	13460	13470	13480	13490	13500
CAGAAAAAAA	GGATCTCAAG	AAGATCCTTT	GATCTTTTCT	ACGGGGTCTG	ACGCTCAGTG
13510	13520	13530	13540	13550	13560
GAACGAAAAC	TCACGTAAAG	GGATTTTGGT	CATGAGATTA	TCAAAAAGGA	TCTTCACCTA
13570	13580	13590	13600	13610	13620
GATCCTTTTA	AATTAAAAAT	GAAGTTTTAA	ATCAATCTAA	AGTATATATG	AGTAAACTTG
13630	13640	13650	13660	13670	13680
GTCTGACAGT	TACCAATGCT	TAATCAGTGA	GGCACCTATC	TCAGCGATCT	GTCTATTTCTG
13690	13700	13710	13720	13730	13740
TTCATCCATA	GTTGCCTGAC	TCCCCGTCGT	GTAGATAACT	ACGATACGGG	AGGGCTTACC
13750	13760	13770	13780	13790	13800
CTGGCCCC	AGTGCTGCAA	TGATACCGCG	AGACCCACGC	TCACCGGCTC	CAGATTTATC
13810	13820	13830	13840	13850	13860
AGCAATAAAC	CAGCCAGCCG	GAAGGGCCGA	GCGCAGAAGT	GGTCTGCAA	CTTTATCCGC
13870	13880	13890	13900	13910	13920
CTCCATCCAG	TCTATTAATT	GTTGCCGGGA	AGCTAGAGTA	AGTAGTTCCG	CAGTTAATAG
13930	13940	13950	13960	13970	13980
TTTGCGCAAC	GTTGTTGCCA	TTGCTGCAGG	CATCGTGGTG	TCACGCTCGT	CGTTTGGTAT
13990	14000	14010	14020	14030	14040
GGCTTCATTC	AGCTCCGGTT	CCCAACGATC	AAGGCGAGTT	ACATGATCCC	CCATGTTGTG
14050	14060	14070	14080	14090	14100
CAAAAAAGCG	GTTAGCTCCT	TCGGTCCTCC	GATCGTTGTC	AGAAGTAAGT	TGGCCGCAGT
14110	14120	14130	14140	14150	14160
GTTATCACTC	ATGGTTATGG	CAGCACTGCA	TAATTCTCTT	ACTGTCATGC	CATCCGTAAG
14170	14180	14190	14200	14210	14220
ATGCTTTTCT	GTGACTGGTG	AGTACTCAAC	CAAGTCATTC	TGAGAATAGT	GTATGCGGGC
14230	14240	14250	14260	14270	14280
ACCGAGTTGC	TCTTGCCCCG	CGTCAACACG	GGATAATACC	GCGCCACATA	GCAGAACTTT
14290	14300	14310	14320	14330	14340

DNASIS  
Desmond rk

AAAAGTGCTC ATCATTGGAA AACGTTCTTC GGGGCGAAAA CTCTCAAGGA TCTTACCGCT  
14350 14360 14370 14380 14390 14400  
GTTGAGATCC AGTTCGATGT AACCCACTCG TGCACCCAAC TGATCTTCAG CATCTTTTAC  
14410 14420 14430 14440 14450 14460  
TTTCACCAGC GTTCTGCGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA AAAAGGGAAT  
14470 14480 14490 14500 14510 14520  
AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT TTTCAATATT ATTGAAGCAT  
14530 14540 14550 14560 14570 14580  
TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA TGTATTTAGA AAAATAAACA  
14590 14600 14610 14620 14630 14640  
AATAGGGGTT CCGCGCACAT TTCCCCGAAA AGTGCCACCT GACGTCTAAG AAACCATTAT  
14650 14660 14670 14680 14690 14700  
TATCATGACA TTAACCTATA AAAATAGGCG TATCACGAGG CCCTTTCGTC TTCAAGAA..

FIGURE 8

2/3/98

DNASIS  
Molly

```

      10      20      30      40      50      60
TTAATTAAGG GCGGAGAAT GGGCGGAAT GGGCGGAGTT AGGGGCGGGA TGGGCGGAGT

      70      80      90     100     110     120
TAGGGGCGGG ACTATGGTTG CTGACTAATT GAGATGCATG CTTTGCATAC TTCTGCCTGC

      130     140     150     160     170     180
TGGGGAGCCT GGGGACTTTC CACACCTGGT TGCTGACTAA TTGAGATGCA TGCTTTGCAT

      190     200     210     220     230     240
ACTTCTGCCT GCTGGGGAGC CTGGGGACTT TCCACACCCT AACTGACACA CATTCCACAG

      250     260     270     280     290     300
AATTAATTCC CCTAGTTATT AATAGTAATC AATTACGGGG TCATTAGTTC ATAGCCCAT

      310     320     330     340     350     360
TATGGAGTTC CGCGTTACAT AACTTACGGT AAATGGCCCG CCTGGCTGAC CGCCCAACGA

      370     380     390     400     410     420
:CCGCCCA TTGACGTCAA TAATGACGTA TGTTCCCAT GTAACGCCAA TAGGGACTTT

      430     440     450     460     470     480
-CCATTGACGT CAATGGGTGG AGTATTTACG GTAAACTGCC CACTTGGCAG TACATCAAGT

      490     500     510     520     530     540
GTATCATATG CCAAGTACGC CCCCTATTGA CGTCAATGAC GGTAAATGGC CCGCCTGGCA

      550     560     570     580     590     600
TTATGCCCAG TACATGACCT TATGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT

      610     620     630     640     650     660
CATCGCTATT ACCATGGTGA TGGGGTTTTG GCAGTACATC AATGGGCGTG GATAGCGGTT

      670     680     690     700     710     720
TGA CTCACGG GGATTTCCAA GTCTCCACCC CATTGACGTC AATGGGAGTT TGTTTTGAAG

      730     740     750     760     770     780
TGGCCGGC CAGCTTTATT TAACGTGTTT ACGTCGAGTC AATTGTACAC TAACGACAGT

      790     800     810     820     830     840
GATGAAAGAA ATACAAAAGC GCATAATATT TTGAACGACG TCGAACCTTT ATTACAAAAC

      850     860     870     880     890     900
AAAACACAAA CGAATATCGA CAAAGCTAGA TTGCTGCTAC AAGATTTGGC AAGTTTTGTG

      910     920     930     940     950     960
GCGTTGAGCG AAAATCCATT AGATAGTCCA GCCATCGGTT CGGAAAAACA ACCCTTGTTT

      970     980     990    1000    1010    1020
GAAACTAATC GAAACCTATT TTACAAATCT ATTGAGGATT TAATATTTAA ATTCAGATAT

      1030    1040    1050    1060    1070    1080
AAAGACGCTG AAAATCATT GATTTTCGCT CTAACATACC ACCCTAAAGA TTATAAATTT

      1090    1100    1110    1120    1130    1140
AATGAATTAT TAAAATACAT CAGCAACTAT ATATTGATAG ACATTTCCAG TTTGTGATAT

      1150    1160    1170    1180    1190    1200
TAGTTTGTGC GTCTCATTAC AATGGCTGTT ATTTTAAACA ACAAACAAC TCTCGCAGAC

      1210    1220    1230    1240    1250    1260
AATAGTATAG AAAAGGGAGG TGAACGTGTT TTGTTTAACG GTTCGTACAA CATTTTGGAA

      1270    1280    1290    1300    1310    1320
AGTTATGTTA ATCCGGTGCT GCTAAAAAAT GGTGTAATTG AACTAGAAGA AGCTGCGTAC

```

DNASIS  
Molly

1330	1340	1350	1360	1370	1380
TATGCCGGCA	ACATATTGTA	CAAAACCGAC	GATCCCAAAT	TCATTGATTA	TATAAAATTTA
1390	1400	1410	1420	1430	1440
ATAATTAAAG	CAACACACTC	CGAAGAACTA	CCAGAAAATA	GCACTGTTGT	AAATTACAGA
1450	1460	1470	1480	1490	1500
AAAACATATGC	GCAGCGGTAC	TATACACCCC	ATTAATAAAG	ACATATATAT	TTATGACAAC
1510	1520	1530	1540	1550	1560
AAAAAATTTA	CTCTATACGA	TAGATACATA	TATGGATACG	ATAATAACTA	TGTTAATTTT
1570	1580	1590	1600	1610	1620
TATGAGGAGA	AAAATGAAAA	AGAGAAGGAA	TACGAAGAAG	AAGACGACAA	GGCGTCTAGT
1630	1640	1650	1660	1670	1680
TTATGTGAAA	ATAAAATTAT	ATTGTCGCAA	ATTAACCTGTG	AATCATTTGA	AAATGATTTT
1690	1700	1710	1720	1730	1740
AAATATTACC	TCAGCGATTA	TAACCTACGCG	TTTTCAATTA	TAGATAATAC	TACAAATGTT
1750	1760	1770	1780	1790	1800
CTTGTTGCGT	TTGGTTTGTA	TCGTTAATAA	AAAACAAATT	TGACATTTAT	AATTGTTTTA
1810	1820	1830	1840	1850	1860
TTATTCAATA	ATTACAAATA	GGATTGAGAC	CCTTGCACTT	GCCAGCAAAC	GGACAGAGCT
1870	1880	1890	1900	1910	1920
TGTCGAGGAG	AGTTGTTGAT	TCATTGTTTG	CCTCCCTGCT	GCGGTTTTTC	ACCGAAGTTC
1930	1940	1950	1960	1970	1980
ATGCCAGTCC	AGCGTTTTTG	CAGCAGAAAA	GCCGCCGACT	TCGGTTTGCG	GTCGCGAGTG
1990	2000	2010	2020	2030	2040
AAGATCCCTT	TCTTGTTACC	GCCAACGCGC	AATATGCCTT	GCGAGGTCGC	AAAATCGGCG
2050	2060	2070	2080	2090	2100
AAATTCCATA	CCTGTTCCAC	GACGACGGCG	CTGACGCGAT	CAAAGACGCG	GTGATACATA
2110	2120	2130	2140	2150	2160
TCCAGCCATG	CACACTGATA	CTCTTCACTC	CACATGTCGG	TGTACATTGA	GTGCAGCCCC
2170	2180	2190	2200	2210	2220
GCTAACGTAT	CCACGCCGTA	TTCGGTGATG	ATAATCGGCT	GATGCAGTTT	CTCCTGCCAG
2230	2240	2250	2260	2270	2280
GCCAGAAAGT	CTTTTCCAG	TACCTTCTCT	GCCGTTTCCA	AATCGCCGCT	TTGGACATAC
2290	2300	2310	2320	2330	2340
CATCCGTAAT	AACGGTTCAG	GCACAGCACA	TCAAAGAGAT	CGCTGATGGT	ATCGGTGTGA
2350	2360	2370	2380	2390	2400
GCGTCGCAGA	ACATTACATT	GACGCAGGTG	ATCGGACGCG	TCGGGTCGAG	TTTACGCGTT
2410	2420	2430	2440	2450	2460
GCTTCCGCCA	GTGGCGCGAA	ATATTCCCGT	GCACCTTGCG	GACGGGTATC	CGGTTTCGTTG
2470	2480	2490	2500	2510	2520
GCAATACTCC	ACATCACCAC	GCTTGGGTGG	TTTTTGTCAC	GCGCTATCAG	CTCTTTAATC
2530	2540	2550	2560	2570	2580
GCCTGTAAGT	GCGCTTGCTG	AGTTTCCCCG	TTGACTGCCT	CTTCGCTGTA	CAGTTCITTC
2590	2600	2610	2620	2630	2640

DNASIS  
Molly. L

GGCTTGTTGC CCGCTTCGAA ACCAATGCCT AAAGAGAGGT TAAAGCCGAC AGCAGCAGTT

2650 2660 2670 2680 2690 2700  
TCATCAATCA CCACGATGCC ATGTTTCATCT GCCCAGTCGA GCATCTCTTC AGCGTAAGGG

2710 2720 2730 2740 2750 2760  
TAATGCGAGG TACGGTAGGA GTTGGCCCCA ATCCAGTCCA TTAATGCGTG GTCGTGCACC

2770 2780 2790 2800 2810 2820  
ATCAGCACGT TATCGAATCC TTTGCCACGC AAGTCCGCAT CTTTCATGACG ACCAAAGCCA

2830 2840 2850 2860 2870 2880  
GTAAAGTAGA ACGGTTTGTG GTTAATCAGG AACTGTTTCG CTTTCACTGC CACTGACCGG

2890 2900 2910 2920 2930 2940  
ATGCCGACGC GAAGCGGGTA GATATCACAC TCTGTCTGGC TTTTGGCTGT GACGCACAGT

2950 2960 2970 2980 2990 3000  
TTATAGAGAT AACCTTCACC CGGTTGCCAG AGGTGCGGAT TCACCACTTG CAAAGTCCCC

3010 3020 3030 3040 3050 3060  
CTAGTGCCCTT GTCCAGTTGC AACCACCTGT TGATCCGCAT CACGCAGTTC AACGCTGACA

3070 3080 3090 3100 3110 3120  
TCACCATTGG CCACCACCTG CCAGTCAACA GACGCGTGGT TACAGTCTTG CGCGACATGC

3130 3140 3150 3160 3170 3180  
GTCACCACGG TGATATCGTC CACCCAGGTG TTCGGCGTGG TGTAAGCAT TACGTGCGA

3190 3200 3210 3220 3230 3240  
TGGATTCCGG CATAGTTAAA GAAATCATGG AAGTAAGACT GCTTTTTCTT GCCGTTTTCG

3250 3260 3270 3280 3290 3300  
TCGGTAATCA CCATTCCCGG CGGGATAGTC TGCCAGTTCA GTTCGTTGTT CACACAAACG

3310 3320 3330 3340 3350 3360  
TTGATACCCC TCGACGGATT AAAGACTTCA AGCGGTCAAC TATGAAGAAG TGTTCGTCTT

3370 3380 3390 3400 3410 3420  
CGTCCCAGTA AGCTATGTCT CCAGAATGTA GCCATCCATC CTTGTCAATC AAGGCGTTGG

3430 3440 3450 3460 3470 3480  
TCGCTTCCGG ATTGTTTACA TAACCGGACA TAATCATAGG TCCTCTGACA CATAATTGCG

3490 3500 3510 3520 3530 3540  
CTCTCTGATT AACGCCCAGC GTTTTCCCGG TATCCAGATC CACAACCTTC GCTTCAAAAA

3550 3560 3570 3580 3590 3600  
ATGGAACAAC TTTACCGACC GCGCCCGGTT TATCATCCCC CTCGGGTGTA ATCAGAATAG

3610 3620 3630 3640 3650 3660  
CTGATGTAGT CTCAGTGAGC CCATATCCTT GTCGTATCCC TGGAAGATGG AAGCGTTTTG

3670 3680 3690 3700 3710 3720  
CAACCGCTTC CCCGACTTCT TTCGAAAGAG GTGCGCCCCC AGAAGCAATT TCGTGTAAT

3730 3740 3750 3760 3770 3780  
TAGATAAATC GTATTTGTCA ATCAGAGTGC TTTTGGCGAA GAATGAAAAT AGGGTTGGTA

3790 3800 3810 3820 3830 3840  
CTAGCAACGC ACTTTGAATT TTGTAATCCT GAAGGGATCG TAAAAACAGC TCTTCTTCAA

3850 3860 3870 3880 3890 3900  
ATCTATACAT TAAGACGACT CGAAATCCAC ATATCAAATA TCCGAGTGTA GTAAACATTC

DNASIS  
Molly.

```

3910      3920      3930      3940      3950      3960
CAAAACCGTG ATGGAATGGA ACAACACTTA AAATCGCAGT ATCCGGAATG ATTTGATTGC

3970      3980      3990      4000      4010      4020
CAAAAATAGG ATCTCTGGCA TGGGAGAATC TGACGCAGGC AGTTCTATGC GGAAGGGCCA

4030      4040      4050      4060      4070      4080
CACCCTTAGG TAACCCAGTA GATCCAGAGG AATTGTTTTG TCACGATCAA AGGACTCTGG

4090      4100      4110      4120      4130      4140
TACAAAATCG TATTCATTAA AACCGGGAGG TAGATGAGAT GTGACGAACG TGTACATCGA

4150      4160      4170      4180      4190      4200
CTGAAATCCC TGGTAATCCG TTTTAGAATC CATGATAATA ATTTTCTGGA TTATTGGTAA

4210      4220      4230      4240      4250      4260
TTTTTTTTCG ACGTTCAAAA TTTTTTGCAA CCCCTTTTTCG GAAACAAACA CTACGGTAGG

4270      4280      4290      4300      4310      4320
TCGAAATG TTCATACTGT TGAGCAATTC ACGTTCATTA TAAATGTCGT TCGCGGGCGC

4330      4340      4350      4360      4370      4380
AACTGCAACT CCGATAAATA ACGCGCCCAA CACCGGCATA AAGAATTGAA GAGAGTTTTC

4390      4400      4410      4420      4430      4440
ACTGCATACG ACGATTCTGT GATTGTATT CAGCCCATAT CGTTTCATAG CTTCTGCCAA

4450      4460      4470      4480      4490      4500
CCGAACGGAC ATTTCGAAGT ATTCCGCGTA CGTGATGTTC ACCTCGATAT GTGCATCTGT

4510      4520      4530      4540      4550      4560
AAAAGGAATT GTTCCAGGAA CCAGGGCGTA TCTCTTCATA GCCTTATGCA GTTGCTCTCC

4570      4580      4590      4600      4610      4620
AGCGGTTCCA TCCTCTAGCT TTGCTTCTCA ATTTCTTATT TGCATAATGA GAAAAAAGG

4630      4640      4650      4660      4670      4680
IATTAATT TTAACACCAA TTCAGTAGTT GATTGAGCAA ATGCGTTGCC AAAAAGGATG

4690      4700      4710      4720      4730      4740
CTTTAGAGAC AGTGTTCTCT GCACAGATAA GGACAAACAT TATTCAGAGG GAGTACCCAG

4750      4760      4770      4780      4790      4800
AGCTGAGACT CCTAAGCCAG TGAGTGGCAC AGCATCCAGG GAGAAATATG CTTGTCATCA

4810      4820      4830      4840      4850      4860
CCGAAGCCTG ATTCCGTAGA GCCACACCCT GGTAAGGGCC AATCTGCTCA CACAGGATAG

4870      4880      4890      4900      4910      4920
AGAGGGCAGG AGCCAGGGCA GAGCATATAA GGTGAGGTAG GATCAGTTGC TCCTCACATT

4930      4940      4950      4960      4970      4980
TGCTTCTGAC ATAGTTGTGT TGGGAGCTTG GATCGATCCA CCATGGGCTT CAATACCTG

4990      5000      5010      5020      5030      5040
ATTGACTGGA ACAGCTGTAG CCCTGAACAG CAGCGTGGC TGCTGACGCG TCCGGCGATT

5050      5060      5070      5080      5090      5100
TCCGCCCTCG ACAGTATTAC CCGGACGGTC AGCGATATTC TGGATAATGT AAAAACGCGC

5110      5120      5130      5140      5150      5160
GGTGACGATG CCCTGCGTGA ATACAGCGCT AAATTTGATA AAACAGAAGT GACAGCGCTA

5170      5180      5190      5200      5210      5220
CGCGTCACCC CTGAAGAGAT CGCCGCCGCC GGC GCGCGTC TGAGCGACGA ATTAAACAG

```

DNASIS  
Molly

5230 5240 5250 5260 5270 5280  
 GCGATGACCG CTGCCGTCAA AAATATTGAA ACGTTCCATT CCGCGCAGAC GCTACCGCTT  
 5290 5300 5310 5320 5330 5340  
 GTAGATGTGG AAACCCAGCC AGGCGTGCGT TGCCAGCAGG TTACGCGTCC CGTCTCGTCT  
 5350 5360 5370 5380 5390 5400  
 GTCGGTCTGT ATATTCCTGG CGGCTCGGCT CCGCTCTTCT CAACGGTGCT GATGCTGGCG  
 5410 5420 5430 5440 5450 5460  
 ACGCCGGCGC GCATTGCGGG ATGCCAGAAG GTGTTTCTGT GCTCGCCGCC GCCCATCGCT  
 5470 5480 5490 5500 5510 5520  
 GATGAAATCC TCTATGCGGC GCAACTGTGT GGCCTGCAGG AAATCTTTAA CGTCGGCGGC  
 5530 5540 5550 5560 5570 5580  
 GCGCAGGCGA TTGCCGCTCT GGCCTTCGGC AGCGAGTCCG TACCGAAAGT GGATAAAATT  
 5590 5600 5610 5620 5630 5640  
 .TGGCCCCG GCAACGCCTT TGTAACCGAA GCCAAACGTC AGGTCAGCCA GCGTCTCGAC  
 5650 5660 5670 5680 5690 5700  
 GGCGCGGCTA TCGATATGCC AGCCGGGCGG TCTGAAGTAC TGGTGATCGC AGACAGCGGC  
 5710 5720 5730 5740 5750 5760  
 GCAACACCGG ATTCGTGCGC TTCTGACCTG CTCTCCAGG CTGAGCACGG CCCGGATTCC  
 5770 5780 5790 5800 5810 5820  
 CAGGTGATCC TGCTGACGCC TGATGCTGAC ATTGCCCGCA AGGTGGCGGA GGCGGTAGAA  
 5830 5840 5850 5860 5870 5880  
 CGTCAACTGG CGGAACTGCC GCGCGCGGAC ACCGCCCGGC AGGCCCTGAG CGCCAGTCGT  
 5890 5900 5910 5920 5930 5940  
 CTGATTGTGA CCAAAGATTT AGCGCAGTGC GTCGCCATCT CTAATCAGTA TGGGCCGGAA  
 5950 5960 5970 5980 5990 6000  
 .ACTTAATCA TCCAGACGCG CAATGCGCGC GATTTGGTGG ATGCGATTAC CAGCGCAGGC  
 6010 6020 6030 6040 6050 6060  
 TCGGTATTTT TCGGCGACTG GTCGCCGGAA TCCGCCGGTG ATTACGCTTC CGGAACCAAC  
 6070 6080 6090 6100 6110 6120  
 CATGTTTTAC CGACCTATGG CTATACTGCT ACCTGTTCCA GCCTTGGGTT AGCGGATTTC  
 6130 6140 6150 6160 6170 6180  
 CAGAAACGGA TGACCGTTCA GGAAGTGTG AAAGCGGGCT TTTCCGCTCT GGCATCAACC  
 6190 6200 6210 6220 6230 6240  
 ATTGAAACAT TGGCGGCGGC AGAACGTCTG ACCGCCATA AAAATGCCGT GACCCTGCGC  
 6250 6260 6270 6280 6290 6300  
 GTAAACGCCC TCAAGGAGCA AGCATGAGGC ACTGAAAACA CTCTCAGCGT CGCTGACTTA  
 6310 6320 6330 6340 6350 6360  
 GCCCGTGAAA ATGTCCGCAA CCTGGAGATC CAGACATGAT AAGATACATT GATGAGTTTG  
 6370 6380 6390 6400 6410 6420  
 GACAAACCAC AACTAGAATG CAGTGAAAAA AATGCTTTAT TTGTGAAATT TGTGATGCTA  
 6430 6440 6450 6460 6470 6480  
 TTGCTTTATT TGTAACCATT ATAAGCTGCA ATAAACAAGT TAACAACAAC AATTGCATTC  
 6490 6500 6510 6520 6530 6540

DNASIS  
Molly L

ATTTTATGTT TCAGGTTTCAG GGGGAGGTGT GGGAGGTTTT TTAAAGCAAG TAAAACCTCT  
 6550 6560 6570 6580 6590 -6600  
 ACAAATGTGG TATGGCTGAT TATGATCTCT AGGGCCGGCC CTCGACGGCG CGCCTCTAGA  
 6610 6620 6630 6640 6650 6660  
 GCAGTGTGGT TTTGCAAGAG GAAGCAAAAA GCCTCTCCAC CCAGGCCTGG AATGTTTCCA  
 6670 6680 6690 6700 6710 6720  
 CCCAATGTCTG AGCAGTGTGG TTTTGCAAGA GGAAGCAAAA AGCCTCTCCA CCCAGGCCTG  
 6730 6740 6750 6760 6770 6780  
 GAATGTTTCC ACCCAATGTC GAGCAAACCC CGCCCAGCGT CTTGTCTATTG GCGAATTCCA  
 6790 6800 6810 6820 6830 6840  
 ACACGCAGAT GCAGTCGGGG CGGCGCGGTC CCAGGTCCAC TTCGCATATT AAGGTGACGC  
 6850 6860 6870 6880 6890 6900  
 CTGTGGCCTC GAACACCGAG CGACCCTGCA GCCAATATGG GATCGGCCAT TGAACAAGAT  
 6910 6920 6930 6940 6950 6960  
 GGATTGCACG CAGGTTCTCC GGCCGCTTGG GTGGAGAGGC TATTCGGCTA TGAATGGGCA  
 6970 6980 6990 7000 7010 7020  
 CAACAGACAA TCGGCTGCTC TGATGCCGCC GTGTTCCGGC TGTCAGCGCA GGGGCGCCCC  
 7030 7040 7050 7060 7070 7080  
 GTTCTTTTGG TCAAGACCGA CCTGTCCGGT GCCCTGAATG AACTGCAGGT AAGTGGCGCC  
 7090 7100 7110 7120 7130 7140  
 GTCGATGGCC GAGGCGGCCT CGGCCTCTGC ATAAATAAAA AAAATTAGTC AGCCATGCAT  
 7150 7160 7170 7180 7190 7200  
 GGGGCGGAGA ATGGGCGGAA CTGGGCGGAG TTAGGGGCGG GATGGGCGGA GTTAGGGGCG  
 7210 7220 7230 7240 7250 7260  
 TGAATATGGT TGCTGACTAA TTGAGATGCA TGCTTTGCAT ACTTCTGCCT GCTGGGGAGC  
 7270 7280 7290 7300 7310 7320  
 CTGGGGACTT TCCACACCTG GTTGCTGACT AATTGAGATG CATGCTTTCG ATACTTCTGC  
 7330 7340 7350 7360 7370 7380  
 CTGCTGGGGA GCCTGGGGAC TTTCCACACC CTAATGACA CACATTCCAC AGAATTAATT  
 7390 7400 7410 7420 7430 7440  
 CCCCTAGTTA TTAATAGTAA TCAATTACGG GGTCATTAGT TCATAGCCCA TATATGGAGT  
 7450 7460 7470 7480 7490 7500  
 TCCGCGTTAC ATAACCTACG GTAAATGGCC CGCCTGGCTG ACCGCCCAAC GACCCCCGCC  
 7510 7520 7530 7540 7550 7560  
 CATTGACGTC AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC  
 7570 7580 7590 7600 7610 7620  
 GTCAATGGGT GGAATATTTA CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATCATA  
 7630 7640 7650 7660 7670 7680  
 TGCCAAGTAC GCCCCCTATT GACGTCAATG ACGGTAAATG GCGCGCCTGG CATTATGCCC  
 7690 7700 7710 7720 7730 7740  
 AGTACATGAC CTTATGGGAC TTTCCTACTT GGCAGTACAT CTACGTATTA GTCATCGCTA  
 7750 7760 7770 7780 7790 7800  
 TTACCATGGT GATGCGGTTT TGGCAGTACA TCAATGGGCG TGGATAGCGG TTTGACTCAC

DNASIS  
Molly L

7810 7820 7830 7840 7850 7860  
GGGGATTTC AAGTCTCCAC CCCATTGACG TCAATGGGAG TTTGTTTTGG CACCAAAATC

7870 7880 7890 7900 7910 7920  
AACGGGACTT TCCAAAATGT CGTAACAACT CCGCCCCATT GACGCAAATG GGCGGTAGGC

7930 7940 7950 7960 7970 7980  
GTGTACGGTG GGAGGTCTAT ATAAGCAGAG CTGGGTACGT GAACCGTCAG ATCGCCTGGA

7990 8000 8010 8020 8030 8040  
GACGCCATCA CAGATCTCTC ACTATGGATT TTCAGGTGCA GATTATCAGC TTCTGTCTAA

8050 8060 8070 8080 8090 8100  
TCAGTGCTTC AGTCATAATG TCCAGAGGAC AAATTGTTCT CTCCCAGTCT CCAGCAATCC

8110 8120 8130 8140 8150 8160  
TGTCTGCATC TCCAGGGGAG AAGGTCACAA TGAATTGCAG GGCCAGCTCA AGTGTAAGTT

8170 8180 8190 8200 8210 8220  
ATCCACTG GTTCCAGCAG AAGCCAGGAT CCTCCCCCAA ACCCTGGATT TATGCCACAT

8230 8240 8250 8260 8270 8280  
CCAACCTGGC TTCTGGAGTC CCTGTTGCT TCACTGGCAG TGGGTCTGGG ACTTCTTACT

8290 8300 8310 8320 8330 8340  
CTCTCACAAT CAGCAGAGTG GAGGTGAAG ATGCTGCCAC TTATTACTGC CAGCAGTGGA

8350 8360 8370 8380 8390 8400  
CTAGTAACCC ACCCAGTTC GGAGGGGGGA CCAAGCTGGA AATCAAACGT ACGGTGGCTG

8410 8420 8430 8440 8450 8460  
CACCATCTGT CTTTCATCTC CCGCCATCTG ATGAGCAGTT GAAATCTGGA ACTGCCTCTG

8470 8480 8490 8500 8510 8520  
TTGTGTGCCT GCTGAATAAC TTCTATCCA GAGAGGCCAA AGTACAGTGG AAGGTGGATA

8530 8540 8550 8560 8570 8580  
TGCCCTCCA ATCGGGTAAC TCCCAGGAGA GTGTACAGA GCAGGACAGC AAGGACAGCA

8590 8600 8610 8620 8630 8640  
CCTACAGCCT CAGCAGCACC CTGACGCTGA GCAAAGCAGA CTACGAGAAA CACAAAGTCT

8650 8660 8670 8680 8690 8700  
ACGCCTGCGA AGTCACCCAT CAGGGCCTGA GCTCGCCCGT CACAAAGAGC TTCAACAGGG

8710 8720 8730 8740 8750 8760  
GAGAGTGTTG AATTCAGATC CGTTAACGGT TACCAACTAC CTAGACTGGA TTCGTGACAA

8770 8780 8790 8800 8810 8820  
CATGCGGCCG TGATATCTAC GTATGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT

8830 8840 8850 8860 8870 8880  
CTGTTGTTTG CCCCTCCCC GTGCCTTCT TGACCCTGGA AGGTGCCACT CCCACTGTCC

8890 8900 8910 8920 8930 8940  
TTTCCTAATA AAATGAGGAA ATTGCATCGC ATTGTCTGAG TAGGTGTCAT TCTATTCTGG

8950 8960 8970 8980 8990 9000  
GGGGTGGGGT GGGGCAGGAC AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG

9010 9020 9030 9040 9050 9060  
GGGATGCGGT GGGCTCTATG GAACCAGCTG GGGCTCGACA GCTATGCCAA GTACGCCCCC

9070 9080 9090 9100 9110 9120  
TATTGACGTC AATGACGGTA AATGGCCCCG CTGGCATTAT GCCCAGTACA TGACCTTATG

DNASIS  
Molly k

```

          9130          9140          9150          9160          9170          9180
GGACTTTCCT ACTTGGCAGT ACATCTACGT ATTAGTCATC GCTATTACCA TGGTGATGCG

          9190          9200          9210          9220          9230          9240
GTTTTGGCAG TACATCAATG GGCCTGGATA GCGGTTTGAC TCACGGGGAT TTCCAAGTCT

          9250          9260          9270          9280          9290          9300
CCACCCCATC GACGTCAATG GGAGTTTGTT TTGGCACCAA AATCAACGGG ACTTCCAAA

          9310          9320          9330          9340          9350          9360
ATGTCGTAAC AACTCCGCCC CATTGACGCA AATGGGCGGT AGGCGTGTAC GGTGGGAGGT

          9370          9380          9390          9400          9410          9420
CTATATAAGC AGAGCTGGGT ACGTCCTCAC ATTCAGTGAT CAGCACTGAA CACAGACCCG

          9430          9440          9450          9460          9470          9480
TCGACATGGG TTGGAGCCTC ATCTTGCTCT TCCTTGTCGC TGTGCTACG CGTGCTCTGT

          9490          9500          9510          9520          9530          9540
CCCAGGTACA ACTGCAGCAG CCTGGGGCTG AGCTGGTGAA GCCTGGGGCC TCAGTGAAGA

          9550          9560          9570          9580          9590          9600
TGTCCTGCAA GGCTTCTGGC TACACATTTA CCAGTTACAA TATGCACTGG GTAAAACAGA

          9610          9620          9630          9640          9650          9660
CACCTGGTCG GGGCCTGGAA TGGATTGGAG CTATTTATCC CGGAAATGGT GATACTTCTT

          9670          9680          9690          9700          9710          9720
ACAATCAGAA GTTCAAAGGC AAGGCCACAT TGACTGCAGA CAAATCCTCC AGCACAGCCT

          9730          9740          9750          9760          9770          9780
ACATGCAGCT CAGCAGCCTG ACATCTGAGG ACTCTGCGGT CTATTACTGT GCAAGATCGA

          9790          9800          9810          9820          9830          9840
CTTACTACGG CCGTGACTGG TACTTCAATG TCTGGGGCGC AGGGACCACG GTCACCGTCT

          9850          9860          9870          9880          9890          9900
CTGCAGCTAG CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT

          9910          9920          9930          9940          9950          9960
CTGGGGGGCAG AGCGGGCCCTG GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG

          9970          9980          9990          10000          10010          10020
TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGACAC CTTCCCGGCT GTCCTACAGT

          10030          10040          10050          10060          10070          10080
CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACCC

          10090          10100          10110          10120          10130          10140
AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTGGAC AAGAAAGCAG

          10150          10160          10170          10180          10190          10200
AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCACCCTG CCCAGCACCT GAACTCCTGG

          10210          10220          10230          10240          10250          10260
GGGGACCGTC AGTCTTCCTC TTCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA

          10270          10280          10290          10300          10310          10320
CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA

          10330          10340          10350          10360          10370          10380
ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT

          10390          10400          10410          10420          10430          10440

```

DNASIS  
Molly

ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT GCACCAGGAC TGGCTGAATG

10450 10460 10470 10480 10490 10500  
GCAAGGAGTA CAAGTGCAAG GTCTCAACA AAGCCCTCCC AGCCCCATC GAGAAAAACA

10510 10520 10530 10540 10550 10560  
TCTCCAAAGC CAAAGGGCAG CCCCAGAAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG

10570 10580 10590 10600 10610 10620  
ATGAGCTGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG

10630 10640 10650 10660 10670 10680  
ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC

10690 10700 10710 10720 10730 10740  
CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA GCTCACCCTG GACAAGAGCA

10750 10760 10770 10780 10790 10800  
CTGGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT

10810 10820 10830 10840 10850 10860  
ACACGCAGAA GAGCCTCTCC CTGTCTCCGG GTAAATGAGG ATCCGTTAAC GGTACCAAC

10870 10880 10890 10900 10910 10920  
TACCTAGACT GGATTCTGTA CAACATGCGG CCGTGATATC TACGTATGAT CAGCCTCGAC

10930 10940 10950 10960 10970 10980  
TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT

10990 11000 11010 11020 11030 11040  
GGAAGGTGCC ACTCCCACTG TCCTTTCCTA ATAAATGAG GAAATTGCAT CGCATTGTCT

11050 11060 11070 11080 11090 11100  
GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG GGGAGGATTG

11110 11120 11130 11140 11150 11160  
TGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT ATGGAACCAG CTGGGGCTCG

11170 11180 11190 11200 11210 11220  
ACAGCAACGC TAGGTCGAGG CCGCTACTAA CTCTCTCCTC CCTCCTTTT CCTGCAGGAC

11230 11240 11250 11260 11270 11280  
GAGGCAGCGC GGCTATCGTG GCTGGCCACG ACGGGCGTTC CTTGCGCAGC TGTGCTCGAC

11290 11300 11310 11320 11330 11340  
GTTGTCACTG AAGCGGGAAG GGACTGGCTG CTATTGGGCG AAGTGCCGGG GCAGGATCTC

11350 11360 11370 11380 11390 11400  
CTGTCATCTC ACCTTGCTCC TGCCGAGAAA GTATCCATCA TGGCTGATGC AATGCGGCGG

11410 11420 11430 11440 11450 11460  
CTGCATACGC TTGATCCGGC TACCTGCCCC TTCGACCACC AAGCGAAACA TCGCATCGAG

11470 11480 11490 11500 11510 11520  
CGAGCACGTA CTCGGATGGA AGCCGGTCTT GTCGATCAGG ATGATCTGGA CGAAGAGCAT

11530 11540 11550 11560 11570 11580  
CAGGGGCTCG CGCCAGCCGA ACTGTTCCGC AGGTAAGTGA GCTCCAATTC AAGCTTCCTA

11590 11600 11610 11620 11630 11640  
GGGCGGCCAG CTAGTAGCTT TGCTTCTCAA TTTCTTATT GCATAATGAG AAAAAAGGA

11650 11660 11670 11680 11690 11700  
AAATTAATTT TAACACCAAT TCAGTAGTTG ATTGAGCAAA TGCCTTGCCA AAAAGGATGC

DNASIS  
Molly

11710	11720	11730	11740	11750	11760
TTTAGAGACA	GTGTTCTCTG	CACAGATAAG	GACAAACATT	ATTCAGAGGG	AGTACCCAGA
11770	11780	11790	11800	11810	11820
GCTGAGACTC	CTAAGCCAGT	GAGTGGCACA	GCATCCAGGG	AGAAATATGC	TTGTCATCAC
11830	11840	11850	11860	11870	11880
CGAAGCCTGA	TTCCGTAGAG	CCACACCCTG	GTAAGGGCCA	ATCTGCTCAC	ACAGGATAGA
11890	11900	11910	11920	11930	11940
GAGGGCAGGA	GCCAGGGCAG	AGCATATAAG	GTGAGGTAGG	ATCAGTTGCT	CCTCACATTT
11950	11960	11970	11980	11990	12000
GCTTCTGACA	TAGTTGTGTT	GGGAGCTTGG	ATAGCTTGGG	GGGGGGACAG	CTCAGGGCTG
12010	12020	12030	12040	12050	12060
CGATTTTCGCG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	CTGGTAGGAT	TTTATCCCCG
12070	12080	12090	12100	12110	12120
GCCATCAT	GGTTCGACCA	TTGAACTGCA	TCGTCGCCGT	GTCCCAAAT	ATGGGGATTG
12130	12140	12150	12160	12170	12180
GCAAGAACGG	AGACCTACCC	TGGCCTCCGC	TCAGGAACGA	GTTCAAGTAC	TTCCAAAGAA
12190	12200	12210	12220	12230	12240
TGACCACAAC	CTCTTCAGTG	GAAGGTAAAC	AGAATCTGGT	GATTATGGGT	AGGAAAACCT
12250	12260	12270	12280	12290	12300
GGTTCCTCCAT	TCCTGAGAAG	AATCGACCTT	TAAAGGACAG	AATTAATATA	GTTCTCAGTA
12310	12320	12330	12340	12350	12360
GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	ATTTTCTTGC	CAAAGTTTG	GATGATGCCT
12370	12380	12390	12400	12410	12420
TAAGACTTAT	TGAACAACCG	GAATTGGCAA	GTAAAGTAGA	CATGGTTTGG	ATAGTCGGAG
12430	12440	12450	12460	12470	12480
AGTTCTGT	TTACCAGGAA	GCCATGAATC	AACCAGGCCA	CCTCAGACTC	TTTGTGACAA
12490	12500	12510	12520	12530	12540
GGATCATGCA	GGAATTTGAA	AGTGACACGT	TTTTCCAGCA	AATTGATTTG	GGGAAATATA
12550	12560	12570	12580	12590	12600
AACTTCTCCC	AGAATACCCA	GGCGTCCTCT	CTGAGGTCCA	GGAGGAAAAA	GGCATCAAGT
12610	12620	12630	12640	12650	12660
ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	TGCTTTCAAG	TTCTCTGCTC
12670	12680	12690	12700	12710	12720
CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT	TTGCTGGCTT	TAGATCAGCC
12730	12740	12750	12760	12770	12780
TCGACTGTGC	CTTCTAGTTG	CCAGCCATCT	GTTGTTTGCC	CCTCCCCCGT	GCCTTCCTTG
12790	12800	12810	12820	12830	12840
ACCCTGGAAG	GTGCCACTCC	CACTGTCCTT	TCCTAATAAA	ATGAGGAAAT	TGCATCGCAT
12850	12860	12870	12880	12890	12900
TGTCTGAGTA	GGTGTCAATC	TATTCTGGGG	GGTGGGGTGG	GGCAGGACAG	CAAGGGGGAG
12910	12920	12930	12940	12950	12960
GATTGGGAAG	ACAATAGCAG	GCATGCTGGG	GATGCGGTGG	GCTCTATGGC	TTCTGAGGGC
12970	12980	12990	13000	13010	13020
GAAAGAACCA	GCTGGGGCTC	GAAGCGGCCG	CCCATTTTCG	TGGTGGTCAG	ATGCGGGATG

DNASIS  
Molly L

13030	13040	13050	13060	13070	13080
GCGTGGGACG	CGGCGGGGAG	CGTCACACTG	AGGTTTTCCG	CCAGACGCCA	CTGCTGCCAG
13090	13100	13110	13120	13130	13140
GCGCTGATGT	GCCCCGGCTT	TGACCATGCG	GTCGCGTTTC	GTTGCACTAC	GCGTACTGTG
13150	13160	13170	13180	13190	13200
AGCCAGAGTT	GCCCCGGCGCT	CTCCGGCTGC	GGTAGTTTCAG	GCAGTTCAAT	CAACTGTTTA
13210	13220	13230	13240	13250	13260
CCTTGTGGAG	CGACATCCAG	AGGCATTCA	CCGCTTGCCA	GCGGCTTACC	ATCCAGCGCC
13270	13280	13290	13300	13310	13320
ACCATCCAGT	GCAGGAGCTC	GTTATCGCTA	TGACGGAACA	GGTATTCGCT	GGTCACTTCG
13330	13340	13350	13360	13370	13380
ATGGTTTGCC	CGGATAAACG	GAACTGGAAA	AACTGCTGCT	GGTGTITTC	TTCCGTCAGC
13390	13400	13410	13420	13430	13440
C .GGATGCG	GCGTGCGGTC	GGCAAAGACC	AGACCGTTCA	TACAGAACTG	GCGATCGTTC
13450	13460	13470	13480	13490	13500
GCGGTATCGC	CAAAATCACC	GCCGTAAGCC	GACCACGGGT	TGCCGTTTTT	ATCATATTTA
13510	13520	13530	13540	13550	13560
ATCAGCGACT	GATCCACCCA	GTCCAGACG	AAGCCGCCCT	GTAAACGGGG	ATACTGACGA
13570	13580	13590	13600	13610	13620
AACGCCTGCC	AGTATTTAGC	GAAACCGCCA	AGACTGTTAC	CCATCGCGTG	GCGGTATTTCG
13630	13640	13650	13660	13670	13680
CAAAGGATCA	GCGGGCGCGT	CTCTCCAGGT	AGCGAAAGCC	ATTTTTTGAT	GGACCATTTT
13690	13700	13710	13720	13730	13740
GGCACAGCCG	GGAAGGGCTG	GTCTTCATCC	ACGCGCGCGT	ACATCGGGCA	AATAATATCG
13750	13760	13770	13780	13790	13800
C .GGCCGTGG	TGTCGGCTCC	GCCGCCTTCA	TACTGCACCG	GGCGGGAAGG	ATCGACAGAT
13810	13820	13830	13840	13850	13860
TTGATCCAGC	GATACAGCGC	GTCGTGATTA	GCGCCGTGGC	CTGATTCATT	CCCCAGCGAC
13870	13880	13890	13900	13910	13920
CAGATGATCA	CACTCGGGTG	ATTACGATCG	CGCTGCACCA	TTGCGGTTAC	GCGTTCGCTC
13930	13940	13950	13960	13970	13980
ATCGCCGGTA	GCCAGCGCGG	ATCATCGGTC	AGACGATTCA	TTGGCACCAT	GCCGTGGGTT
13990	14000	14010	14020	14030	14040
TCAATATTGG	CTTCATCCAC	CACATACAGG	CCGTAGCGGT	CGCACAGCGT	GTACCACAGC
14050	14060	14070	14080	14090	14100
GGATGGTTTC	GATAATGCGA	ACAGCGCACG	GCGTTAAAGT	TGTTCTGCTT	CATCAGCAGG
14110	14120	14130	14140	14150	14160
ATATCCTGCA	CCATCGTCTG	CTCATCCATG	ACCTGACCAT	GCAGAGGATG	ATGCTCGTGA
14170	14180	14190	14200	14210	14220
CGGTAAACGC	CTCGAATCAG	CAACGGCTTG	CCGTTCAGCA	GCAGCAGACC	ATTTTCAATC
14230	14240	14250	14260	14270	14280
CGCACCTCGC	GGAAACCGAC	ATCGCAGGCT	TCTGCTTCAA	TCAGCGTGCC	GTCGGCGGGT
14290	14300	14310	14320	14330	14340

DNASIS  
Molly L

TGCAGTTCAA CCACCGCACG ATAGAGATTC GGGATTTTCGG CGCTCCACAG TTTCGGGTTT

14350 14360 14370 14380 14390 14400  
TCGACGTTCA GACGTAGTGT GACGCGATCG GCATAACCAC CACGCTCATC GATAATTTCA14410 14420 14430 14440 14450 14460  
CCGCCGAAAG GCGCGGTGCC GCTGGCGACC TCGGTTTCAC CCTGCCATAA AGAAACTGTT14470 14480 14490 14500 14510 14520  
ACCCGTAGGT AGTCACGCAA CTCGCCGCAC ATCTGAACTT CAGCCTCCAG TACAGCGCGG14530 14540 14550 14560 14570 14580  
CTGAAATCAT CATTAAAGCG AGTGGAACA TGGAAATCGC TGATTGTGT AGTCGGTTTA14590 14600 14610 14620 14630 14640  
TGCAGCAACG AGACGTCACG GAAAATGCCG CTCATCCGCC ACATATCTG ATCTTCCAGA14650 14660 14670 14680 14690 14700  
TAACTGCCGT CACTCCAGCG CAGCACCATC ACCGCGAGGC GGTTCCTCC GCGCGGTAAA14710 14720 14730 14740 14750 14760  
AATGCGCTCA GGTCAAATTC AGACGGCAA CGACTGTCCT GGCGTAACC GACCCAGCGC14770 14780 14790 14800 14810 14820  
CCGTTGCACC ACAGATGAAA CGCCGAGTTA ACGCCATCAA AAATAATTCG CGTCTGGCCT14830 14840 14850 14860 14870 14880  
TCCTGTAGCC AGCTTTCATC AACATTAAAT GTGAGCGAGT AACAACCCGT CGGATTCTCC14890 14900 14910 14920 14930 14940  
GTGGGAACAA ACGGCGGATT GACCGTAATG GGATAGGTGA CGTTGGTGTA GATGGGCGCA14950 14960 14970 14980 14990 15000  
TCGTAACCGT GCATCTGCCA GTTTGAGGGG ACGACGACAG TATCGGCTC AGGAAGATCG15010 15020 15030 15040 15050 15060  
CACTCCAGCC AGCTTTCGG CACCGTTCT GGTGCCGGAA ACCAGGCAA GCGCCATTCG15070 15080 15090 15100 15110 15120  
CCATTCAGGC TGCGCAACTG TTGGGAAGGG CGATCGGTGC GGGCCTCTC GCTATTACGC15130 15140 15150 15160 15170 15180  
CAGCTGGCGA AAGGGGGATG TGCTGCAAGG CGATTAAGTT GGGTAACGCC AGGTTTTC15190 15200 15210 15220 15230 15240  
CAGTCACGAC GTTGTAACAC GACTTAATCC GTCGAGGGG TGCTCGAAG CAGACGACCT15250 15260 15270 15280 15290 15300  
TCCGTTGTGC AGCCAGCGGC GCCTGCGCCG GTGCCACAA TCGTGCGCA ACAAACTAAA15310 15320 15330 15340 15350 15360  
CCAGAACAAA TTATACCGGC GGCACCGCCG CCACCACCTT CTCCCGTGCC TAACATTCCA15370 15380 15390 15400 15410 15420  
GCGCCTCCAC CACCACCACC ACCATCGATG TCTGAATTGC CGCCCCTCC ACCAATGCCG15430 15440 15450 15460 15470 15480  
ACGGAACCTC AACCCGCTGC ACCTTAGAC GACAGACAAC AATTGTTGGA AGCTATTAGA15490 15500 15510 15520 15530 15540  
AACGAAAAA ATCGCACTCG TCTCAGACCG GTCAAACCAA AACGCGGCC CGAAACCAGT15550 15560 15570 15580 15590 15600  
ACAATAGTTG AGGTGCCGAC TGTGTTGCCT AAAGAGACAT TTGAGCCTAA ACCGCCGTCT

DNASIS  
Molly L

15610 15620 15630 15640 15650 15660  
GCATCACC GC CACCACCTCC GCCTCCGCCT CCGCCGCCAG CCCCCCCTGC GCCTCCACCG

15670 15680 15690 15700 15710 15720  
ATGGTAGATT TATCATCAGC TCCACCACCG CCGCCATTAG TAGATTGCCC GTCTGAAATG

15730 15740 15750 15760 15770 15780  
TTACCACCGC CTGCACCATC GCTTTCTAAC GTGTTGTCTG AATTAAAAATC GGGCACAGTT

15790 15800 15810 15820 15830 15840  
AGATTGAAAC CCGCCCAAAA ACGCCCGCAA TCAGAAATAA TTCCAAAAAG CTCAACTACA

15850 15860 15870 15880 15890 15900  
AATTTGATCG CGGACGTGTT AGCCGACACA ATTAATAGGC GTCGTGTGGC TATGGCAAAA

15910 15920 15930 15940 15950 15960  
TCGTCTTCGG AAGCAACTTC TAACGACGAG GGTGGGACG ACGACGATAA TCGGCCTAAT

15970 15980 15990 16000 16010 16020  
AGCTAACA CGCCCGATGT TAAATATGTC CAAGCTACTA GTGGTACCGC TTGGCAGAAC

16030 16040 16050 16060 16070 16080  
ATATCCATCG CGTCCGCCAT CTCCAGCAGC CGCAGCGGC GCATCTCGGG CAGCGTTGGG

16090 16100 16110 16120 16130 16140  
TCCTGGCCAC GGGTGCGCAT GATCGTGCTC CTGTCGTTGA GGACCCGGCT AGGCTGGCGG

16150 16160 16170 16180 16190 16200  
GGTTGCCTTA CTGGTTAGCA GAATGAATCA CCGATACGCG AGCGAACGTG AAGCGACTGC

16210 16220 16230 16240 16250 16260  
TGCTGCAAAA CGTCTGCGAC CTGAGCAACA ACATGAATGG TCTTCGGTTT CCGTGTTTCG

16270 16280 16290 16300 16310 16320  
TAAAGTCTGG AAACGCGGAA GTCAGCGCCC TGCACCATTG TGTTCGGGAT CTGCATCGCA

16330 16340 16350 16360 16370 16380  
GATGCTGCT GGCTACCCTG TGGAACACCT ACATCTGTAT TAACGAAGCG CTGGCATTGA

16390 16400 16410 16420 16430 16440  
CCCTGAGTGA TTTTCTCTG GTCCCGCCGC ATCCATACCG CCAGTTGTTT ACCCTCACAA

16450 16460 16470 16480 16490 16500  
CGTTCCAGTA ACCGGGCATG TTCATCATCA GTAACCCGTA TCGTGAGCAT CCTCTCTCGT

16510 16520 16530 16540 16550 16560  
TTCATCGGTA TCATTACCCC CATGAACAGA AATCCCCCTT ACACGGAGGC ATCAGTGACC

16570 16580 16590 16600 16610 16620  
AAACAGGAAA AAACCGCCCT TAACATGGCC CGCTTTATCA GAAGCCAGAC ATTAACGCTT

16630 16640 16650 16660 16670 16680  
CTGGAGAAAC TCAACGAGCT GGACGCGGAT GAACAGGCAG ACATCTGTGA ATCGCTTCAC

16690 16700 16710 16720 16730 16740  
GACCACGCTG ATGAGCTTTA CCGCAGCTGC CTCGCGCGTT TCGGTGATGA CCGTGAAAAC

16750 16760 16770 16780 16790 16800  
CTCTGACACA TGCAGTCCC GGAGACGGTC ACAGCTTGTC TGTAAGCGGA TGCCGGGAGC

16810 16820 16830 16840 16850 16860  
AGACAAGCCC GTCAGGGCGC GTCAGCGGGT GTTGGCGGGT GTCGGGGCGC AGCCATGACC

16870 16880 16890 16900 16910 16920  
CAGTCACGTA GCGATAGCGG AGTGTATACT GGCTTAACTA TGCGGCATCA GAGCAGATTG

DNASIS  
Molly

```

16930      16940      16950      16960      16970      16980
TACTGAGAGT GCACCATATG CCGTGTGAAA TACCGCACAG ATGCGTAAGG AGAAAATACC

16990      17000      17010      17020      17030      17040
GCATCAGGCG CTCTTCCGCT TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC

17050      17060      17070      17080      17090      17100
GGCGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT ATCCACAGAA TCAGGGGATA

17110      17120      17130      17140      17150      17160
ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC CAGGAACCGT AAAAAGGCCG

17170      17180      17190      17200      17210      17220
CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC CCCCTGACGA GCATCACAAA AATCGACGCT

17230      17240      17250      17260      17270      17280
CAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCTGGAA

17290      17300      17310      17320      17330      17340
GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC

17350      17360      17370      17380      17390      17400
TCCCTTCGGG AAGCGTGGCG CTTTCTCATA GCTCACGCTG TAGGTATCTC AGTTCGGTGT

17410      17420      17430      17440      17450      17460
AGGTCGTTTG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC CGTTCAGCCC GACCGCTGCG

17470      17480      17490      17500      17510      17520
CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG ACACGACTTA TCGCCACTGG

17530      17540      17550      17560      17570      17580
CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT

17590      17600      17610      17620      17630      17640
TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC

17650      17660      17670      17680      17690      17700
TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA CAAACCACCG

17710      17720      17730      17740      17750      17760
CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC GCGCAGAAAA AAAGGATCTC

17770      17780      17790      17800      17810      17820
AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA GTGGAACGAA AACTCACGTT

17830      17840      17850      17860      17870      17880
AAGGGATTTT GGTCA TGAGA TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA

17890      17900      17910      17920      17930      17940
AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT

17950      17960      17970      17980      17990      18000
GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC ATAGTTGCCT

18010      18020      18030      18040      18050      18060
GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGGCTT ACCATCTGGC CCCAGTGCTG

18070      18080      18090      18100      18110      18120
CAATGATACC GCGAGACCCA CGCTCACC GG CTCCAGATTT ATCAGCAATA AACCAGCCAG

18130      18140      18150      18160      18170      18180
CCGGAAGGGC CGAGCGCAGA AGTGGTCTCT CAACTTTATC CGCCTCCATC CAGTCTATTA

18190      18200      18210      18220      18230      18240

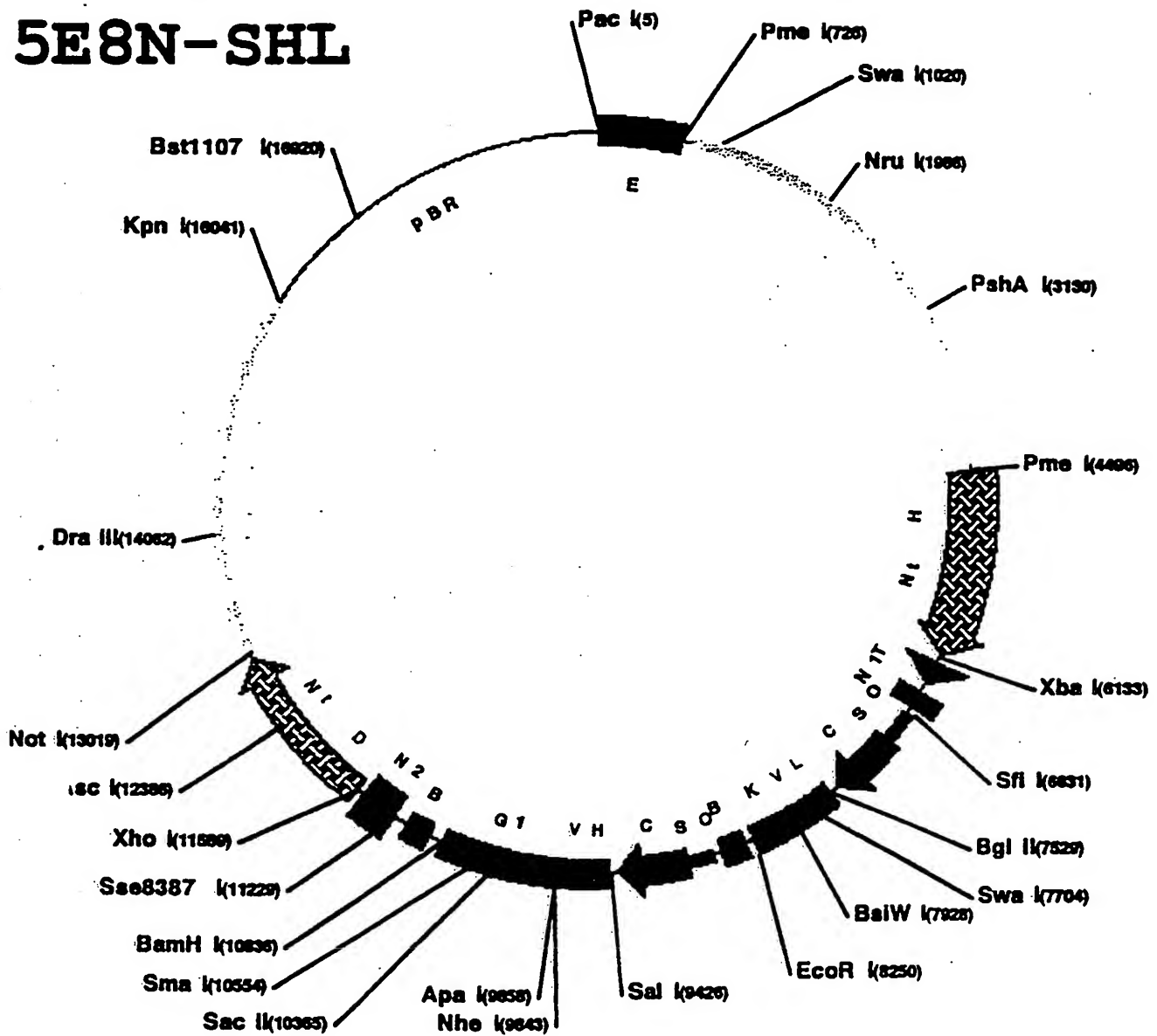
```

DNASIS  
Molly Lusk

ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC AACGTTGTTG  
 18250 18260 18270 18280 18290 18300  
 CCATTGCTGC AGGCATCGTG GTGTCACGCT CGTCGTTTGG TATGGCTTCA TTCAGCTCCG  
 18310 18320 18330 18340 18350 18360  
 GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT GTGCAAAAAA GCGGTTAGCT  
 18370 18380 18390 18400 18410 18420  
 CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC AGTGTTATCA CTCATGGTTA  
 18430 18440 18450 18460 18470 18480  
 TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG  
 18490 18500 18510 18520 18530 18540  
 GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC  
 18550 18560 18570 18580 18590 18600  
 TGGCGTCAAC ACGGGATAAT ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG CTCATCATTG  
 18610 18620 18630 18640 18650 18660  
 GAAAACGTTT TTCGGGGCGA AAACCTCTCA GGATCTTACC GCTGTTGAGA TCCAGTTCTGA  
 18670 18680 18690 18700 18710 18720  
 TGTAAACCCAC TCGTGACCC AACTGATCTT CAGCATCTTT TACTTTCACC AGCGTTTCTG  
 18730 18740 18750 18760 18770 18780  
 GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT  
 18790 18800 18810 18820 18830 18840  
 GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG CATTATCAG GGTATTGTC  
 18850 18860 18870 18880 18890 18900  
 TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG GTTCCGCGCA  
 18910 18920 18930 18940 18950 18960  
 TTTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT TATTATCATG ACATTAACCT  
 18970 18980 18990 19000 19010 19020  
 ATAAAAATAG GCGTATCAG AGGCCCTTTC GTCTTCAAGA A.....

FIGURE 9

# Mandy + 5E8N-SHL



- Nt D = Inactive Dihydrofolate reductase  
 E = CMV and SV40 enhancers  
 Nt H = Inactive *Salmonella* Histidinol Dehydrogenase  
 T = Herpes Simplex thymidine kinase promoter and polyoma enhancer  
 C = Cytomegalovirus promoter/enhancer  
 B = Bovine growth hormone polyadenylation  
 N1 = Neomycin phosphotransferase exon 1  
 M2 = Neomycin phosphotransferase exon 2  
 K = Human kappa constant  
 G1 = Human Gamma 1 constant  
 VL = Variable light chain anti-CD23 primate 5E8 and leader  
 VH = Variable heavy chain anti-CD23 primate 5E8N- and leader  
 SO = SV40 Origin of replication

Mandy cut XbaI Xho I and ligated to Xba I Xho I fragment from XKG1+CD23 5E8N-SHL

## FIGURE 10

DNASIS  
Mandy E8N-SHL

```

      10          20          30          40          50          60
TTAATTAAGG GCGCGAGAAT GGGCGGAAC TGGCGGAGTT AGGGGCGGGA TGGGCGGAGT

      70          80          90         100         110         120
TAGGGGCGGG ACTATGGTTG CTGACTAATT GAGATGCATG CTTTGCATAC TTCTGCCTGC

      130         140         150         160         170         180
TGGGGAGCCT GGGGACTTTC CACACCTGGT TGCTGACTAA TTGAGATGCA TGCTTTGCAT

      190         200         210         220         230         240
ACTTCTGCCT GCTGGGGAGC CTGGGGACTT TCCACACCCT AACTGACACA CATTCCACAG

      250         260         270         280         290         300
AATTAATTCC CCTAGTTATT AATAGTAATC AATTACGGGG TCATTAGTTC ATAGCCCAT

      310         320         330         340         350         360
TATGGAGTTC CGCGTTACAT AACTTACGGT AAATGGCCCG CCTGGCTGAC CGCCCAACGA

      370         380         390         400         410         420
CCCCGCCCA TTGACGTCAA TAATGACGTA TGTTCCCAT GTAACGCCAA TAGGGACTTT

      430         440         450         460         470         480
CCATTGACGT CAATGGGTGG AGTATTTACG GTAAACTGCC CACTTGGCAG TACATCAAGT

      490         500         510         520         530         540
GTATCATATG CCAAGTACGC CCCCTATTGA CGTCAATGAC GGTAATGGC CCGCCTGGCA

      550         560         570         580         590         600
TTATGCCCAG TACATGACCT TATGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT

      610         620         630         640         650         660
CATCGCTATT ACCATGGTGA TCGGTTTTTG GCAGTACATC AATGGGCGTG GATAGCGGTT

      670         680         690         700         710         720
TGA CTCACGG GGATTTCAA GTCTCCACCC CATTGACGTC AATGGGAGTT TGT TTTGAAG

      730         740         750         760         770         780
GT TTAAC AGCTTGGCCG GCCAGCTTTA TTTAACGTGT TTACGTCGAG TCAATTGTAC

      790         800         810         820         830         840
ACTAACGACA GTGATGAAAG AAATACAAAA GCGCATAATA TTTTGAACGA CGTCGAACCT

      850         860         870         880         890         900
TTATTACAAA ACAAACACA AACGAATATC GACAAAGCTA GATTGCTGCT ACAAGATTTG

      910         920         930         940         950         960
GCAAGTTTTG TGGCGTTGAG CGAAATCCA TTAGATAGTC CAGCCATCGG TTCGGAAAAA

      970         980         990        1000        1010        1020
CAACCCTTGT TTGAAACTAA TCGAAACCTA TTTTACAAAT CTATTGAGGA TTTAATATTT

      1030        1040        1050        1060        1070        1080
AAATTCAGAT ATAAAGACGC TGAAATCAT TTGATTTTCG CTCTAACATA CCACCCTAAA

      1090        1100        1110        1120        1130        1140
GATTATAAAT TTAATGAATT ATTAATAATC ATCAGCAACT ATATATTGAT AGACATTTC

      1150        1160        1170        1180        1190        1200
AGTTTGTGAT ATTAGTTTGT GCGTCTCATT ACAATGGCTG TTATTTTAA CAACAAACAA

      1210        1220        1230        1240        1250        1260
CTGCTCGCAG ACAATAGTAT AGAAAAGGGA GGTGAACTGT TTTTGT TAA CGGTTTCGTAC

      1270        1280        1290        1300        1310        1320
AACATTTTGG AAAGTTATGT TAATCCGGTG CTGCTAAAAA ATGGTGTAAT TGAAC TAGAA

```

DNASIS  
Mandy SE8N-SHL

```

1330      1340      1350      1360      1370      1380
GAAGCTGCGT ACTATGCCGG CAACATATTG TACAAAACCG ACGATCCCAA ATTCATTGAT

1390      1400      1410      1420      1430      1440
TATATAAATT TAATAATTAA AGCAACACAC TCCGAAGAAC TACCAGAAAA TAGCACTGTT

1450      1460      1470      1480      1490      1500
GTAAATTACA GAAAAACTAT GCGCAGCGGT ACTATACACC CCATTAAAAA AGACATATAT

1510      1520      1530      1540      1550      1560
ATTTATGACA ACAAAAAATT TACTCTATAC GATAGATACA TATATGGATA CGATAATAAC

1570      1580      1590      1600      1610      1620
TATGTTAATT TTTATGAGGA GAAAAATGAA AAAGAGAAGG AATACGAAGA AGAAGACGAC

1630      1640      1650      1660      1670      1680
AAGGCGTCTA GTTTATGTGA AAATAAAATT ATATTGTCGC AAATTAAGTG TGAATCATTT

1690      1700      1710      1720      1730      1740
GAAAATGATT TTAAATATTA CCTCAGCGAT TATAACTACG CGTTTTCAT TATAGATAAT

1750      1760      1770      1780      1790      1800
ACTACAAATG TTCTTGTTGC GTTTGGTTTG TATCGTTAAT AAAAAACAAA TTTGACATTT

1810      1820      1830      1840      1850      1860
ATAATTGTTT TATTATTCAA TAATTACAAA TAGGATTGAG ACCCTTGCGA TTGCCAGCAA

1870      1880      1890      1900      1910      1920
ACGGACAGAG CTTGTCGAGG AGAGTTGTTG ATTCATTGTT TGCCTCCCTG CTGCGGTTTT

1930      1940      1950      1960      1970      1980
TCACCGAAGT TCATGCCAGT CCAGCGTTTT TGCAGCAGAA AAGCCGCCGA CTTCGGTTTG

1990      2000      2010      2020      2030      2040
CGGTCGCGAG TGAAGATCCC TTTCTTGTTA CCGCCAACGC GCAATATGCC TTGCGAGGTC

2050      2060      2070      2080      2090      2100
GCAAAATCGG CGAAATTCCA TACCTGTTCA CCGACGACGG CGCTGACGCG ATCAAAGACG

2110      2120      2130      2140      2150      2160
CGGTGATACA TATCCAGCCA TGCACACTGA TACTCTTCAC TCCACATGTC GGTGTACATT

2170      2180      2190      2200      2210      2220
GAGTGACGCC CGGCTAACGT ATCCACGCCG TATTCGGTGA TGATAATCGG CTGATGCAGT

2230      2240      2250      2260      2270      2280
TTCTCCTGCC AGGCCAGAAG TTCTTTTCC AGTACCTTCT CTGCCGTTTC CAAATCGCCG

2290      2300      2310      2320      2330      2340
CTTTGGACAT ACCATCCGTA ATAACGGTTC AGGCACAGCA CATCAAAGAG ATCGCTGATG

2350      2360      2370      2380      2390      2400
GTATCGGTGT GAGCGTCGCA GAACATTACA TTGACGCAGG TGATCGGACG CGTCGGGTCG

2410      2420      2430      2440      2450      2460
AGTTTACGCG TTGCTTCCGC CAGTGGCGCG AAATATTCCC GTGCACCTTG CGGACGGGTA

2470      2480      2490      2500      2510      2520
TCCGGTTCGT TGGCAATACT CCACATCACC ACGCTTGGGT GGTTTTTGTC ACGCGCTATC

2530      2540      2550      2560      2570      2580
AGCTCTTTAA TCGCCTGTAA GTGCGCTTGC TGAGTTTCCC CGTTGACTGC CTCTTCGCTG

2590      2600      2610      2620      2630      2640

```

DNASIS  
Mandy + SE8N-SHL

TACAGTTCTT TCGGCTTGTT GCCCCTTCG AAACCAATGC CTAAAGAGAG GTTAAAGCCG  
 2650 2660 2670 2680 2690 2700  
 ACAGCAGCAG TTTCATCAAT CACCACGATG CCATGTTTAT CTGCCCAGTC GAGCATCTCT  
 2710 2720 2730 2740 2750 2760  
 TCAGCGTAAG GGTAATGCGA GGTACGGTAG GAGTTGGCCC CAATCCAGTC CATTAAATGCG  
 2770 2780 2790 2800 2810 2820  
 TGGTCGTGCA CCATCAGCAC GTTATCGAAT CCTTTGCCAC GCAAGTCCGC ATCTTCATGA  
 2830 2840 2850 2860 2870 2880  
 CGACCAAAGC CAGTAAAGTA GAACGGTTTG TGGTTAATCA GGAAGTGTTC GCCCTTCACT  
 2890 2900 2910 2920 2930 2940  
 GCCACTGACC GGATGCCGAC GCGAAGCGGG TAGATATCAC ACTCTGTCTG GCTTTTGGCT  
 2950 2960 2970 2980 2990 3000  
 TGACGCACA GTTCATAGAG ATAACCTTCA CCCGGTTGCC AGAGGTGCGG ATTCACCACT  
 3010 3020 3030 3040 3050 3060  
 TGCAAAGTCC CGCTAGTGCC TTGTCCAGTT GCAACCACCT GTTGATCCGC ATCAGCAGT  
 3070 3080 3090 3100 3110 3120  
 TCAACGCTGA CATCACCATT GGCCACCACC TGCCAGTCAA CAGACGCGTG GTTACAGTCT  
 3130 3140 3150 3160 3170 3180  
 TGC GCGACAT GCGTCACCAC GGTGATATCG TCCACCAGG TGTTCCGCGT GGTGTAGAGC  
 3190 3200 3210 3220 3230 3240  
 ATTACGCTGC GATGGATTCC GGCATAGTTA AAGAAATCAT GGAAGTAAGA CTGCTTTTTT  
 3250 3260 3270 3280 3290 3300  
 TTGCCGTTTT CGTCGGTAAT CACCATTCCC GGCGGGATAG TCTGCCAGTT CAGTTCGTTG  
 3310 3320 3330 3340 3350 3360  
 TCACACAAA CGGTGATACC CCTCGACGGA TTAAAGACTT CAAGCGGTCA ACTATGAAGA  
 3370 3380 3390 3400 3410 3420  
 AGTGTTCTGC TTCGTCCAG TAAGCTATGT CTCCAGAAATG TAGCCATCCA TCCTTGTCAA  
 3430 3440 3450 3460 3470 3480  
 TCAAGGCGTT GGTCGCTTCC GGATTGTTTA CATAACCGGA CATAATCATA GGTCCTCTGA  
 3490 3500 3510 3520 3530 3540  
 CACATAATTC GCCTCTCTGA TTAACGCCCA GCGTTTTCCC GGTATCCAGA TCCACAACCT  
 3550 3560 3570 3580 3590 3600  
 TCGCTTCAAA AAATGGAACA ACTTTACCGA CCGCGCCCGG TTTATCATCC CCCTCGGGTG  
 3610 3620 3630 3640 3650 3660  
 TAATCAGAAT AGCTGATGTA GTCTCAGTGA GCCCATATCC TTGTCGTATC CCTGGAAGAT  
 3670 3680 3690 3700 3710 3720  
 GGAAGCGTTT TGCAACCGCT TCCCCGACTT CTTTCGAAAG AGGTGCGCCC CCAGAAGCAA  
 3730 3740 3750 3760 3770 3780  
 TTTCGTGTAA ATTAGATAAA TCGTATTTGT CAATCAGAGT GCTTTTGGCG AAGAATGAAA  
 3790 3800 3810 3820 3830 3840  
 ATAGGGTTGG TACTAGCAAC GCACTTTGAA TTTTGTAATC CTGAAGGGAT CGTAAAAACA  
 3850 3860 3870 3880 3890 3900  
 GCTCTTCTTC AAATCTATAC ATTAAGACGA CTCGAAATCC ACATATCAA TATCCGAGTG

DNASIS  
Mandy + SE8N-SHL

```

3910      3920      3930      3940      3950      3960
TAGTAAACAT TCCAAAACCG TGATGGAATG GAACAACACT TAAAATCGCA GTATCCGGAA

3970      3980      3990      4000      4010      4020
TGATTTGATT GCCAAAAATA GGATCTCTGG CATGCGAGAA TCTGACGCAG GCAGTTCTAT

4030      4040      4050      4060      4070      4080
GCGGAAGGGC CACACCCTTA GGTAACCCAG TAGATCCAGA GGAATTGTTT TGTCACGATC

4090      4100      4110      4120      4130      4140
AAAGGACTCT GGTACAAAAT CGTATTCATT AAAACCGGGA GGTAGATGAG ATGTGACGAA

4150      4160      4170      4180      4190      4200
CGTGTACATC GACTGAAATC CCTGGTAATC CGTTTTAGAA TCCATGATAA TAATTTTCTG

4210      4220      4230      4240      4250      4260
GATTATTGGT AATTTTTTTT GCACGTTCAA AATTTTTTGC AACCCCTTTT TGGAAACAAA

4270      4280      4290      4300      4310      4320
CTACGGTA GGCTGCGAAA TGTTCACTACT GTTGAGCAAT TCACGTTTAT TATAAATGTC

4330      4340      4350      4360      4370      4380
GTTTCGCGGGC GCAACTGCAA CTCCGATAAA TAACGCGCCC AACACCGGCA TAAAGAATTG

4390      4400      4410      4420      4430      4440
AAGAGAGTTT TCACTGCATA CGACGATTCT GTGATTTGTA TTCAGCCCAT ATCGTTTCAT

4450      4460      4470      4480      4490      4500
AGCTTCTGCC AACCGAACGG ACATTTGCAA GTATTCCGCG TACAGCCCGG CCGTTTAAAC

4510      4520      4530      4540      4550      4560
GGCCGGGCTT CAATACCCTG ATTGACTGGA ACAGCTGTAG CCCTGAACAG CAGCGTGCGC

4570      4580      4590      4600      4610      4620
TGCTGACGCG TCCGGCGATT TCCGCCTCTG ACAGTATTAC CCGGACGGTC AGCGATATTC

4630      4640      4650      4660      4670      4680
GATAATGT AAAAACGCGC GGTGACGATG CCCTGCGTGA ATACAGCGCT AAATTTGATA

4690      4700      4710      4720      4730      4740
AAACAGAAGT GACAGCGCTA CGCGTCACCC CTGAAGAGAT CGCCGCCGCC GGC GCGCGTC

4750      4760      4770      4780      4790      4800
TGAGCGACGA ATTA AACAG GCGATGACCG CTGCCGTCAA AAATATTGAA ACGTTCCATT

4810      4820      4830      4840      4850      4860
CCGCGCAGAC GCTACCGCCT GTAGATGTGG AAACCCAGCC AGGCGTGCGT TGCCAGCAGG

4870      4880      4890      4900      4910      4920
TTACGCGTCC CGTCTCGTCT GTCGGTCTGT ATATTCCCGG CGGCTCGGCT CCGCTCTTCT

4930      4940      4950      4960      4970      4980
CAACGGTGCT GATGCTGGCG ACGCCGGCGC GCATTGCGGG ATGCCAGAAG GTGGTTCTGT

4990      5000      5010      5020      5030      5040
GCTCGCCGCC GCCCATCGCT GATGAAATCC TCTATGCGGC GCAACTGTGT GGC GTGCAGG

5050      5060      5070      5080      5090      5100
AAATCTTTAA CGTCGGCGGC GCGCAGGCGA TTGCCGCTCT GGCCTTCGGC AGCGAGTCCG

5110      5120      5130      5140      5150      5160
TACCGAAAGT GGATAAAATT TTTGGCCCCG GCAACGCCTT TGTAACCGAA GCCAAACGTC

5170      5180      5190      5200      5210      5220
AGGTCAGCCA GCGTCTCGAC GGC GCGGCTA TCGATATGCC AGCCGGGCCG TCTGAAGTAC

```

DNASI  
Mandy SE8N-SHL

5230 5240 5250 5260 5270 5280  
TGGTGATCGC AGACAGCGGC GCAACACCGG ATTCGTCGC TTCTGACCTG CTCTCCCAGG

5290 5300 5310 5320 5330 5340  
CTGAGCACGG CCCGGATTCC CAGGTGATCC TGCTGACGCC TGATGCTGAC ATTGCCCCGA

5350 5360 5370 5380 5390 5400  
AGGTGGCGGA GGCGGTAGAA CGTCAACTGG CGGAACTGCC GCGCGCGGAC ACCGCCCCGGC

5410 5420 5430 5440 5450 5460  
AGGCCCTGAG CGCCAGTCGT CTGATTGTGA CCAAAGATT AGCGCAGTGC GTCGCCATCT

5470 5480 5490 5500 5510 5520  
CTAATCAGTA TGGGCCGGAA CACTTAATCA TCCAGACGCG CAATGCGCGC GATTTGGTGG

5530 5540 5550 5560 5570 5580  
ATGCGATTAC CAGCGCAGGC TCGGTATTTT TCGGCGACTG GTCGCCGGAA TCCGCCGGTG

5590 5600 5610 5620 5630 5640  
ATTACGCTTC CGGAACCAAC CATGTTTTAC CGACCTATGG CTATACTGCT ACCTGTTCCA

5650 5660 5670 5680 5690 5700  
GCCTTGGGTT AGCGGATTTT CAGAAACGGA TGACCGTTCA GGAAGTGTG AAAGCGGGCT

5710 5720 5730 5740 5750 5760  
TTTCCGCTCT GGCATCAACC ATTGAAACAT TGGCGGCGGC AGAACGTCTG ACCGCCATA

5770 5780 5790 5800 5810 5820  
AAAATGCCGT GACCTGCGC GTAAACGCCC TCAAGGAGCA AGCATGAGCA CTGAAACAC

5830 5840 5850 5860 5870 5880  
TCTCAGCGTC GCTGACTTAG CCCGTGAAAA TGTCGCAAC CTGGAGATCC AGACATGGAT

5890 5900 5910 5920 5930 5940  
AAGATACATT GATGAGTTTG GACAAACCAC AACTAGAATG CAGTGAAAAA AATGCTTTAT

5950 5960 5970 5980 5990 6000  
TTGTGAAATT TGTGATGCTA TTGCTTTATT TGTAACCATT ATAAGCTGCA ATAAACAAGT

6010 6020 6030 6040 6050 6060  
TAACAACAAC AATTGCATTC ATTTTATGTT TCAGGTTCAG GGGGAGGTGT GGGAGGTTTT

6070 6080 6090 6100 6110 6120  
TTAAAGCAAG TAAACCTCT ACAAATGTGG TATGGCTGAT TATGATCTCT AGGGCCGGCC

6130 6140 6150 6160 6170 6180  
CTCGACGGCG CGTCTAGAGC AGTGTGGTTT TCAAGAGGAA GCAAAAAGCC TCTCCACCCA

6190 6200 6210 6220 6230 6240  
GGCCTGGAAT GTTTCCACCC AATGTCGAGC AGTGTGGTTT TGCAAGAGGA AGCAAAAAGC

6250 6260 6270 6280 6290 6300  
CTCTCCACCC AGGCCTGGAA TGTTCACACC CAATGTCGAG CAAACCCCGC CCAGCGTCTT

6310 6320 6330 6340 6350 6360  
GTCATTGGCG AATTGGAACA CGCATATGCA GTCGGGGCGG CGCGGTCCCA GGTCCACTTC

6370 6380 6390 6400 6410 6420  
GCATATTAAG GTGGCGCGTG TGGCCTCGAA CACCGAGCGA CCCTGCAGCC AATATGGGAT

6430 6440 6450 6460 6470 6480  
CGGCCATTGA ACAAGATGGA TTGCACGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT

6490 6500 6510 6520 6530 6540

DNASIS

Mandy E8N-SHL

TCGGCTATGA CTGGGCACAA CAGACAATCG GCTGCTCTGA TGCCGCCGTG TTCCGGCTGT

6550 6560 6570 6580 6590 6600  
CAGCGCAGGG GCGCCCGTT CTTTTGTCA AGACCGACCT GTCCGGTGCC CTGAATGAAC

6610 6620 6630 6640 6650 6660  
TGCAGGTAAG TGCGGCCGTC GATGGCCGAG GCGGCCTCGG CCTCTGCATA AATAAAAAA

6670 6680 6690 6700 6710 6720  
ATTAGTCAGC CATGCATGGG GCGGAGAATG GGCAGAACTG GCGGGAGTTA GGGGCGGGAT

6730 6740 6750 6760 6770 6780  
GGGCGGAGTT AGGGGCGGGA CTATGGTTGC TGAATAATTG AGATGCATGC TTTGCATACT

6790 6800 6810 6820 6830 6840  
TCTGCCTGCT GGGGAGCCTG GGGACTTTCC ACACCTGGTT GCTGACTAAT TGAGATGCAT

6850 6860 6870 6880 6890 6900  
CTTTGCATA CTTCTGCCTG CTGGGGAGCC TGGGGACTTT CCACACCCTA ACTGACACAC

6910 6920 6930 6940 6950 6960  
ATTCCACAGA ATTAATTCCC CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA

6970 6980 6990 7000 7010 7020  
TAGCCCATAT ATGGAGTTCC GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC

7030 7040 7050 7060 7070 7080  
GCCCAACGAC CCCCGCCCAT TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT

7090 7100 7110 7120 7130 7140  
AGGGACTTTC CATTGACGTC AATGGGTGGA GTATTTACGG TAAACTGCCC ACTTGGCAGT

7150 7160 7170 7180 7190 7200  
ACATCAAGTG TATCATATGC CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC

7210 7220 7230 7240 7250 7260  
GCCTGGCAT TATGCCCAGT ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA

7270 7280 7290 7300 7310 7320  
CGTATTAGTC ATCGCTATTA CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG

7330 7340 7350 7360 7370 7380  
ATAGCGGTTT GACTCACGGG GATTTCCAAG TCTCCACCCC ATTGACGTCA ATGGGAGTTT

7390 7400 7410 7420 7430 7440  
GTTTTGGCAC CAAAATCAAC GGGACTTTCC AAAATGTCGT AACAACTCCG CCCATTGAC

7450 7460 7470 7480 7490 7500  
GCAAATGGGC GGTAGGCGTG TACGGTGGGA GGTCTATATA AGCAGAGCTG GGTACGTGAA

7510 7520 7530 7540 7550 7560  
CCGTCAGATC GCCTGGAGAC GCCATCACAG ATCTCTCACC ATGGACATGA GGGTCCCCGC

7570 7580 7590 7600 7610 7620  
TCAGCTCCTG GGGCTCCTT TGCTCTGGCT CCCAGGTGCC AGATGTGACA TCCAGATGAC

7630 7640 7650 7660 7670 7680  
CCAGTCTCCA TCTTCCCTGT CTGCATCTGT AGGGGACAGA GTCACCATCA CTTGCAGGGC

7690 7700 7710 7720 7730 7740  
AAGTCAGGAC ATTAGGTATT ATTTAAATTG GTATCAGCAG AAACCAGGAA AAGCTCCTAA

7750 7760 7770 7780 7790 7800  
GCTCCTGATC TATGTTGCAT CCAGTTTGCA AAGTGGGGTC CCATCAAGGT TCAGCGGCAG

DNASIS  
Mandy E8N-SHL

```

7810      7820      7830      7840      7850      7860
TGGATCTGGG ACAGAGTTCA CTCTACCGT CAGCAGCCTG CAGCCTGAAG ATTTTGGCAG

7870      7880      7890      7900      7910      7920
TTATTACTGT CTACAGGTTT ATAGTACCCC TCGGACGTTT GGCCAAGGGA CCAAGGTGGA

7930      7940      7950      7960      7970      7980
AATCAAACGT ACGGTGGCTG CACCATCTGT CTTTCATCTT CCGCCATCTG ATGAGCAGTT

7990      8000      8010      8020      8030      8040
GAAATCTGGA ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA

8050      8060      8070      8080      8090      8100
AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCAGGAGA GTGTCACAGA

8110      8120      8130      8140      8150      8160
GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC CTGACGCTGA GCAAAGCAGA

8170      8180      8190      8200      8210      8220
TACGAGAAA CACAAAGTCT ACGCCTGCGA AGTCACCCAT CAGGGCCTGA GCTCGCCCGT

8230      8240      8250      8260      8270      8280
CACAAAGAGC TTCAACAGGG GAGAGTGTTG AATTCAGATC CGTTAACGGT TACCAACTAC

8290      8300      8310      8320      8330      8340
CTAGACTGGA TTCGTGACAA CATGCGGCCG TGATATCTAC GTATGATCAG CCTCGACTGT

8350      8360      8370      8380      8390      8400
GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCTTCCT TGACCCTGGA

8410      8420      8430      8440      8450      8460
AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA ATTGCATCGC ATTGTCTGAG

8470      8480      8490      8500      8510      8520
TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC AGCAAGGGGG AGGATTGGGA

8530      8540      8550      8560      8570      8580
ACAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG GCTTCTGAGG CGGAAAGAAC

8590      8600      8610      8620      8630      8640
CAGCTGGGAC TAGTCGCAAT TGGGCGGAGT TAGGGGCGGG ATGGGCGGAG TTAGGGGCGG

8650      8660      8670      8680      8690      8700
GACTATGGTT GCTGACTAAT TGAGATGCAT GCTTTGCATA CTTCTGCCTG CTGGGGAGCC

8710      8720      8730      8740      8750      8760
TGGGGACTTT CCACACCTGG TTGCTGACTA ATTGAGATGC ATGCTTTGCA TACTTCTGCC

8770      8780      8790      8800      8810      8820
TGCTGGGGAG CCTGGGGACT TTCCACACCC TAACTGACAC ACATTCCACA GAATTAATTC

8830      8840      8850      8860      8870      8880
CCCTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT CATAGCCCAT ATATGGAGTT

8890      8900      8910      8920      8930      8940
CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA CCGCCCAACG ACCCCCGCCC

8950      8960      8970      8980      8990      9000
ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA ATAGGGACTT TCCATTGACG

9010      9020      9030      9040      9050      9060
TCAATGGGTG GAGTATTTAC GGTAAGTGC CCACTTGGCA GTACATCAAG TGTATCATAT

9070      9080      9090      9100      9110      9120
GCCAAGTACG CCCCTATTG ACGTCAATGA CGGTAAATGG CCCGCCTGGC ATTATGCCCA

```

DNASIS  
Mandy SE8N-SHL

9130	9140	9150	9160	9170	9180
GTACATGACC	TTATGGGACT	TTCCTACTTG	GCAGTACATC	TACGTATTAG	TCATCGCTGT
9190	9200	9210	9220	9230	9240
TACCATGGTG	ATGCGGTTTT	GGCAGTACAT	CAATGGGCGT	GGATAGCGGT	TTGACTCAGC
9250	9260	9270	9280	9290	9300
GGGATTTCGA	AGTCTCCACC	CCATTGACGT	CAATGGGAGT	TTGTTTTGGC	ACCAAAATCA
9310	9320	9330	9340	9350	9360
ACGGGACTTT	CCAAAATGTC	GTAACAACTC	CGCCCCATTG	ACGCAAATGG	GCGGTAGGCG
9370	9380	9390	9400	9410	9420
TGTACGGTGG	GAGGTCTATA	TAAGCAGAGC	TGGGTACGTG	AACCGTCAGA	TCGCCTGGAG
9430	9440	9450	9460	9470	9480
ACGCCGTCGA	CATGGGTTGG	AGCCTCATCT	TGCTCTTCCT	TGTCGCTGTT	GCTACGCGTG
9490	9500	9510	9520	9530	9540
CCTGTCCGA	GGTGCACTG	GTGGAGTCTG	GGGGCGGCTT	GGCAAAGCCT	GGGGGGTCCC
9550	9560	9570	9580	9590	9600
TGAGACTCTC	CTGCGCAGCC	TCCGGGTTCA	GGTTCACCTT	CAATAACTAC	TACATGGACT
9610	9620	9630	9640	9650	9660
GGGTCCGCCA	GGCTCCAGGG	CAGGGGCTGG	AGTGGGTCTC	ACGTATTAGT	AGTAGTGGTG
9670	9680	9690	9700	9710	9720
ATCCACATG	GTACGCAGAC	TCCGTGAAGG	GCAGATTCAC	CATCTCCAGA	GAGAACGCCA
9730	9740	9750	9760	9770	9780
AGAACACACT	GTTTCTTCAA	ATGAACAGCC	TGAGAGCTGA	GGACACGGCT	GTCTATTACT
9790	9800	9810	9820	9830	9840
GTGCGAGCTT	GA CTACAGGG	TCTGACTCCT	GGGGCCAGGG	AGTCCTGGTC	ACCGTCTCCT
9850	9860	9870	9880	9890	9900
LAGCTAGCAC	CAAGGGCCCA	TGGGTCTTCC	CCCTGGCACC	CTCCTCCAAG	AGCACCTCTG
9910	9920	9930	9940	9950	9960
GGGGCACAGC	GGCCCTGGGC	TGCCTGGTCA	AGGACTACTT	CCCCGAACCG	GTGACGGTGT
9970	9980	9990	10000	10010	10020
CGTGGAATC	AGGCGCCCTG	ACCAGCGGCG	TGCACACCTT	CCCGGCTGTC	CTACAGTCCT
10030	10040	10050	10060	10070	10080
CAGGACTCTA	CTCCCTCAGC	AGCGTGGTGA	CCGTGCCCTC	CAGCAGCTTG	GGCACCAGA
10090	10100	10110	10120	10130	10140
CCTACATCTG	CAACGTGAAT	CACAAGCCCA	GCAACACCAA	GGTGGACAAG	AAAGTTGAGC
10150	10160	10170	10180	10190	10200
CCAAATCTTG	TGACAAAAT	CACACATGCC	CACCGTGCCC	AGCACCTGAA	CTCCTGGGGG
10210	10220	10230	10240	10250	10260
GACCGTCAGT	CTTCCTCTTC	CCCCCAAAC	CCAAGGACAC	CCTCATGATC	TCCCGGACCC
10270	10280	10290	10300	10310	10320
CTGAGGTCAC	ATGCGTGGTG	GTGGACGTGA	GCCACGAAGA	CCCTGAGGTC	AAGTTCAACT
10330	10340	10350	10360	10370	10380
GGTACGTGGA	CGGCGTGGAG	GTGCATAATG	CCAAGACAAA	GCCGCGGGAG	GAGCAGTACA
10390	10400	10410	10420	10430	10440

DNASIS  
Mandy SE8N-SHL

ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA  
 10450 10460 10470 10480 10490 10500  
 AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCTCCCAGC CCCATCGAG AAAACCATCT  
 10510 10520 10530 10540 10550 10560  
 CCAAAGCCAA AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCCA TCCCGGGATG  
 10570 10580 10590 10600 10610 10620  
 AGCTGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA  
 10630 10640 10650 10660 10670 10680  
 TCGCCGTGGA GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG  
 10690 10700 10710 10720 10730 10740  
 TGCTGGACTC CGACGGCTCC TTCTTCTCT ACAGCAAGCT CACCGTGGAC AAGAGCAGGT  
 10750 10760 10770 10780 10790 10800  
 CGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCCTACA  
 10810 10820 10830 10840 10850 10860  
 CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA AATGAGGATC CGTTAACGGT TACCAACTAC  
 10870 10880 10890 10900 10910 10920  
 CTAGACTGGA TTCGTGACAA CATGCGGCCG TGATATCTAC GTATGATCAG CCTCGACTGT  
 10930 10940 10950 10960 10970 10980  
 GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCC GTGCCTTCTT TGACCCTGGA  
 10990 11000 11010 11020 11030 11040  
 AGGTGCCACT CCCACTGTCC TTTCTAATA AAATGAGGAA ATTGCATCGC ATTGTCTGAG  
 11050 11060 11070 11080 11090 11100  
 TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC AGCAAGGGGG AGGATTGGGA  
 11110 11120 11130 11140 11150 11160  
 AGACAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG GCTTCTGAGG CGGAAAGAAC  
 11170 11180 11190 11200 11210 11220  
 CAGCTGGGGC TCGACAGCAA CGCTAGGTCG AGGCCGCTAC TAACTCTCTC CTCCCTCCTT  
 11230 11240 11250 11260 11270 11280  
 TTTCCTGCAG GACGAGGCAG CGCGGCTATC GTGGCTGGCC ACGACGGGCG TTCCTTGCGC  
 11290 11300 11310 11320 11330 11340  
 AGCTGTGCTC GACGTTGTCA CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC  
 11350 11360 11370 11380 11390 11400  
 GGGGCAGGAT CTCCTGTCTC CTCACCTTGC TCCTGCCGAG AAAGTATCCA TCATGGCTGA  
 11410 11420 11430 11440 11450 11460  
 TGCAATGCGG CGGCTGCATA CGCTTGATCC GGCTACCTGC CCATTCGACC ACCAAGCGAA  
 11470 11480 11490 11500 11510 11520  
 ACATCGCATC GAGCGAGCAC GTACTCGGAT GGAAGCCGGT CTTGTCGATC AGGATGATCT  
 11530 11540 11550 11560 11570 11580  
 GGACGAAGAG CATCAGGGGC TCGCGCCAGC CGAACTGTTT GCCAGGTAAG TGAGCTCCAA  
 11590 11600 11610 11620 11630 11640  
 TTCAAGCTCT CGAGCTAGGG CGGCCAGCTA GTAGCTTTGC TTCTCAATTT CTTATTTGCA  
 11650 11660 11670 11680 11690 11700  
 TAATGAGAAA AAAAGGAAAA TTAATTTTAA CACCAATTCA GTAGTTGATT GAGCAAATGC

DNASIS  
Mandy SE8N-SHL

11710	11720	11730	11740	11750	11760
GTTGCCAAAA	AGGATGCTTT	AGAGACAGTG	TTCTCTGCAC	AGATAAGGAC	AAACATTATT
11770	11780	11790	11800	11810	11820
CAGAGGGAGT	ACCCAGAGCT	GAGACTCCTA	AGCCAGTGAG	TGGCACAGCA	TCCAGGGAGA
11830	11840	11850	11860	11870	11880
AATATGCTTG	TCATCACCGA	AGCCTGATTG	CGTAGAGCCA	CACCCTGGTA	AGGGCCAATC
11890	11900	11910	11920	11930	11940
TGCTCACACA	GGATAGAGAG	GGCAGGAGCC	AGGGCAGAGC	ATATAAGGTG	AGGTAGGATC
11950	11960	11970	11980	11990	12000
AGTTGCTCCT	CACATTTGCT	TCTGACATAG	TTGTGTTGGG	AGCTTGGATA	GCTTGGGGGG
12010	12020	12030	12040	12050	12060
GGGACAGCTC	AGGGCTGCGA	TTTCGCGCCA	AACTTGACGG	CAATCCTAGC	GTGAAGGCTG
12070	12080	12090	12100	12110	12120
AGGATTTT	ATCCCCGCTG	CCATCATGGT	TCGACCATTG	AACTGCATCG	TCGCCGTGTC
12130	12140	12150	12160	12170	12180
CCAAAATATG	GGGATTGGCA	AGAACGGAGA	CCTACCCTGG	CCTCCGCTCA	GGAACGAGTT
12190	12200	12210	12220	12230	12240
CAAGTACTTC	CAAAGAATGA	CCACAACCTC	TTCAGTGGAA	GGTAAACAGA	ATCTGGTGAT
12250	12260	12270	12280	12290	12300
TATGGGTAGG	AAAACCTGGT	TCTCCATTCC	TGAGAAGAAT	CGACCTTTAA	AGGACAGAAT
12310	12320	12330	12340	12350	12360
TAATATAGTT	CTCAGTAGAG	AACTCAAAGA	ACCACCACGA	GGAGCTCATT	TTCTTGCCAA
12370	12380	12390	12400	12410	12420
AAGTTTGGAT	GATGCCTTAA	CGTAGGCGCG	CCATTAAGAC	TTATTGAACA	ACCGGAATTG
12430	12440	12450	12460	12470	12480
CAAGTAAAG	TAGACATGGT	TTGGATAGTC	GGAGGCAGTT	CTGTTTACCA	GGAAGCCATG
12490	12500	12510	12520	12530	12540
AATCAACCAG	GCCACCTCAG	ACTCTTTGTG	ACAAGGATCA	TGCAGGAATT	TGAAAGTGAC
12550	12560	12570	12580	12590	12600
ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	TATAAACTTC	TCCCAGAATA	CCCAGGCGTC
12610	12620	12630	12640	12650	12660
CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	AAGTATAAGT	TTGAAGTCTA	CGAGAAGAAA
12670	12680	12690	12700	12710	12720
GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	GCTCCCTCC	TAAAGCTATG	CATTTTATA
12730	12740	12750	12760	12770	12780
AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	AGCCTCGACT	GTGCCTTCTA	GTTGCCAGCC
12790	12800	12810	12820	12830	12840
ATCTGTTGTT	TGCCCCCTCC	CCGTGCCTTC	CTTGACCCTG	GAAGGTGCCA	CTCCCACTGT
12850	12860	12870	12880	12890	12900
CCTTTCCTAA	TAAATGAGG	AAATTGCATC	GCATTGTCTG	AGTAGGTGTC	ATTCTATTCT
12910	12920	12930	12940	12950	12960
GGGGGGTGGG	GTGGGGCAGG	ACAGCAAGGG	GGAGGATTGG	GAAGACAATA	GCAGGCATGC
12970	12980	12990	13000	13010	13020
TGGGGATGCG	GTGGGGCTCTA	TGGCTTCTGA	GGCGGAAAGA	ACCAGCTGGG	GCTCGAAGCG

DNASIS  
Mandy

E8N-SHL

13030	13040	13050	13060	13070	13080
GCCGCCATT	TCGCTGGTGG	TCAGATGCGG	GATGGCGTGG	GACGCGGCGG	GGAGCGTCAC
13090	13100	13110	13120	13130	13140
ACTGAGGTTT	TCCGCCAGAC	GCCACTGCTG	CCAGGCGCTG	ATGTGCCCCG	CTTCTGACCA
13150	13160	13170	13180	13190	13200
TGCGGTCGCG	TTCGGTTGCA	CTACGCGTAC	TGTGAGCCAG	AGTTGCCCCG	CGCTCTCCGG
13210	13220	13230	13240	13250	13260
CTGCGGTAGT	TCAGGCAGTT	CAATCAACTG	TTTACCTTGT	GGAGCGACAT	CCAGAGGCAC
13270	13280	13290	13300	13310	13320
TTCACCGCTT	GCCAGCGGCT	TACCATCCAG	CGCCACCATC	CAGTGCAGGA	GCTCGTTATC
13330	13340	13350	13360	13370	13380
GCTATGACGG	AACAGGTATT	CGCTGGTCAC	TTCGATGGTT	TGCCCGGATA	AACGGAACTG
13390	13400	13410	13420	13430	13440
AAAACTGC	TGCTGGTGTT	TTGCTTCCGT	CAGCGCTGGA	TGCGGCGTGC	GGTCGGCAAA
13450	13460	13470	13480	13490	13500
GACCAGACCG	TTCATACAGA	ACTGGCGATC	GTTGCGCGTA	TCGCCAAAAT	CACCGCCGTA
13510	13520	13530	13540	13550	13560
AGCCGACCAC	GGGTTGCCGT	TTTCATCATA	TTTAATCAGC	GACTGATCCA	CCCAGTCCCA
13570	13580	13590	13600	13610	13620
GACGAAGCCG	CCCTGTAAAC	GGGGATACTG	ACGAAACGCC	TGCCAGTATT	TAGCGAAACC
13630	13640	13650	13660	13670	13680
GCCAAGACTG	TTACCCATCG	CGTGGGCGTA	TTCGCAAAGG	ATCAGCGGGC	GCGTCTCTCC
13690	13700	13710	13720	13730	13740
AGGTAGCGAA	AGCCATTTTT	TGATGGACCA	TTTCGGCACA	GCCGGGAAGG	GCTGGTCTTC
13750	13760	13770	13780	13790	13800
TCCACGCGC	GCGTACATCG	GGCAAATAAT	ATCGGTGGCC	GTGGTGTCGG	CTCCGCCGCC
13810	13820	13830	13840	13850	13860
TTCATACTGC	ACCGGGCGGG	AAGGATCGAC	AGATTTGATC	CAGCGATACA	GCGCGTCGTG
13870	13880	13890	13900	13910	13920
ATTAGCGCCG	TGGCCTGATT	CATTCGCCAG	CGACCAGATG	ATCACACTCG	GGTGATTACG
13930	13940	13950	13960	13970	13980
ATCGCGCTGC	ACCATTGCGG	TTACGCGTTC	GCTCATCGCC	GGTAGCCAGC	GCGGATCATC
13990	14000	14010	14020	14030	14040
GGTCAGACGA	TTCATTGGCA	CCATGCCGTG	GGTTTCAATA	TTGGCTTCAT	CCACCACATA
14050	14060	14070	14080	14090	14100
CAGGCCGTAG	CGGTGCGACA	GCGTGTACCA	CAGCGGATGG	TTCGGATAAT	GCGAACAGCG
14110	14120	14130	14140	14150	14160
CACGGCGTTA	AAGTTGTTCT	GCTTCATCAG	CAGGATATCC	TGCACCATCG	TCTGCTCATC
14170	14180	14190	14200	14210	14220
CATGACCTGA	CCATGCAGAG	GATGATGCTC	GTGACGGTTA	ACGCCTCGAA	TCAGCAACGG
14230	14240	14250	14260	14270	14280
CTTGCCGTTC	AGCAGCAGCA	GACCATTTTC	AATCCGCACC	TCGCGGAAAC	CGACATCGCA
14290	14300	14310	14320	14330	14340

DNASIS  
Mandy E8N-SHL

GGCTTCTGCT TCAATCAGCG TGCCGTCGGC GGTGTGCAGT TCAACCACCG CACGATAGAG

14350 14360 14370 14380 14390 14400  
ATTCGGGATT TCGGCGCTCC ACAGTTTCGG GTTTTCGACG TTCAGACGTA GTGTGACGGC

14410 14420 14430 14440 14450 14460  
ATCGGCATAA CCACCACGCT CATCGATAAT TTCACCGCCG AAAGGCGCGG TGCCGCTGGC

14470 14480 14490 14500 14510 14520  
GACCTGCGTT TCACCCTGCC ATAAAGAAAC TGTACCCGT AGGTAGTCAC GCAACTCGCC

14530 14540 14550 14560 14570 14580  
GCACATCTGA ACTTCAGCCT CCAGTACAGC GCGGCTGAAA TCATCATTAA AGCGAGTGGC

14590 14600 14610 14620 14630 14640  
AACATGGAAA TCGCTGATTT GTGTAGTCGG TTTATGCAGC AACGAGACGT CACGGAAAAT

14650 14660 14670 14680 14690 14700  
CCCGCTCATC CGCCACATAT CCTGATCTTC CAGATAACTG CCGTCACTCC AGCGCAGCAC

14710 14720 14730 14740 14750 14760  
CATCACCGCG AGGCGGTTTT CTCCGGCGCG TAAAAATGCG CTCAGGTCAA ATTCAGACGG

14770 14780 14790 14800 14810 14820  
CAAACGACTG TCCTGGCCGT AACCGACCCA GCGCCCGTTG CACCACAGAT GAAACGCCGA

14830 14840 14850 14860 14870 14880  
GTAAACGCCA TCAAAAATAA TTCGCGTCTG GCCTTCCTGT AGCCAGCTTT CATCAACATT

14890 14900 14910 14920 14930 14940  
AAATGTGAGC GAGTAACAAC CCGTCGGATT CTCCGTGGGA ACAACGGCG GATTGACCGT

14950 14960 14970 14980 14990 15000  
AATGGGATAG GTCACGTTGG TGTAGATGGG CGCATCGTAA CCGTGATCT GCCAGTTTGA

15010 15020 15030 15040 15050 15060  
CCGGACGACG ACAGTATCGG CCTCAGGAAG ATCGCACTCC AGCCAGCTTT CCGGCACCGC

15070 15080 15090 15100 15110 15120  
TTCTGGTGCC GGAAACCAGG CAAAGCGCCA TTCGCCATTC AGGCTGCGCA ACTGTTGGGA

15130 15140 15150 15160 15170 15180  
AGGGCGATCG GTGCGGGCCT CTTCGCTATT ACGCCAGCTG GCGAAAGGGG GATGTGCTGC

15190 15200 15210 15220 15230 15240  
AAGGCGATTA AGTTGGGTAA CGCCAGGGTT TTCCAGTCA CGACGTTGTA AAACGACTTA

15250 15260 15270 15280 15290 15300  
ATCCGTCGAG GGGCTGCCTC GAAGCAGACG ACCTTCGTT GTGCAGCCAG CGGCGCCTGC

15310 15320 15330 15340 15350 15360  
GCCGGTGCCC ACAATCGTGC GCGAACAAC TAAACCAGAA CAAATTATAC CGGCGGCACC

15370 15380 15390 15400 15410 15420  
GCCGCCACCA CTTCTCTCCG TGCCTAACAT TCCAGCGCCT CCACCACCAC CACCACCATC

15430 15440 15450 15460 15470 15480  
GATGTCTGAA TTGCCGCCCC CTCCACCAAT GCCGACGGAA CCTCAACCCG CTGCACCTTT

15490 15500 15510 15520 15530 15540  
AGACGACAGA CAACAATTGT TGGAAGCTAT TAGAAACGAA AAAAATCGCA CTCGTCTCAG

15550 15560 15570 15580 15590 15600  
ACCGGTCAAA CCAAAAACGG CGCCCGAAAC CAGTACAATA GTTGAGGTGC CGACTGTGTT

DNASIS

Mandy

E8N-SHL

15610	15620	15630	15640	15650	15660
GCCTAAAGAG	ACATTTGAGC	CTAAACCGCC	GTCTGCATCA	CCGCCACCAC	CTCCGCCTCC
15670	15680	15690	15700	15710	15720
GCCTCCGCCG	CCAGCCCCGC	CTGCGCCTCC	ACCGATGGTA	GATTTATCAT	CAGCTCCACC
15730	15740	15750	15760	15770	15780
ACCGCCGCCA	TTAGTAGATT	TGCCGTCTGA	AATGTTACCA	CCGCCTGCAC	CATCGCTTTC
15790	15800	15810	15820	15830	15840
TAACGTGTTG	TCTGAATTAA	AATCGGGCAC	AGTTAGATTG	AAACCCGCCC	AAAAACGCC
15850	15860	15870	15880	15890	15900
GCAATCAGAA	ATAATTCAA	AAAGCTCAAC	TACAAATTG	ATCGCGGACG	TGTTAGCCGA
15910	15920	15930	15940	15950	15960
CACAATTAAT	AGGCGTCGTG	TGGCTATGGC	AAAATCGTCT	TCGGAAGCAA	CTTCTAACGA
15970	15980	15990	16000	16010	16020
AGGGTTGG	GACGACGACG	ATAATCGGCC	TAATAAAGCT	AACACGCCCC	ATGTTAAATA
16030	16040	16050	16060	16070	16080
TGTCCAAGCT	ACTAGTGGTA	CCGCTTGGCA	GAACATATCC	ATCGCGTCCG	CCATCTCCAG
16090	16100	16110	16120	16130	16140
CAGCCGCACG	CGGCGCATCT	CGGGCAGCGT	TGGGTCTGG	CCACGGGTGC	GCATGATCGT
16150	16160	16170	16180	16190	16200
GCTCCTGTCTG	TTGAGGACCC	GGCTAGGCTG	GCGGGGTTGC	CTTACTGGTT	AGCAGAAATGA
16210	16220	16230	16240	16250	16260
ATCACCATA	CGCGAGCGAA	CGTGAAGCGA	CTGCTGCTGC	AAAACGTCTG	CGACCTGAGC
16270	16280	16290	16300	16310	16320
AACAACATGA	ATGGTCTTCG	GTTTCCGTGT	TTCGTAAAGT	CTGGAAACGC	GGAAGTCAGC
16330	16340	16350	16360	16370	16380
CCTGCACC	ATTATGTTCC	GGATCTGCAT	CGCAGGATGC	TGCTGGCTAC	CCTGTGGAAC
16390	16400	16410	16420	16430	16440
ACCTACATCT	GTATTAACGA	AGCGCTGGCA	TTGACCCTGA	GTGATTTTTC	TCTGGTCCCG
16450	16460	16470	16480	16490	16500
CCGCATCCAT	ACCGCCAGTT	GTTTACCCTC	ACAACGTTCC	AGTAACCGGG	CATGTTTCATC
16510	16520	16530	16540	16550	16560
ATCAGTAACC	CGTATCGTGA	GCATCCTCTC	TCGTTTCATC	GGTATCATT	CCCCATGAA
16570	16580	16590	16600	16610	16620
CAGAAATCCC	CCTTACACGG	AGGCATCAGT	GACCAAACAG	GAAAAAACCG	CCCTTAACAT
16630	16640	16650	16660	16670	16680
GGCCCCGCTTT	ATCAGAAGCC	AGACATTAAC	GCTTCTGGAG	AAACTCAACG	AGCTGGACGC
16690	16700	16710	16720	16730	16740
GGATGAACAG	GCAGACATCT	GTGAATCGCT	TCACGACCAC	GCTGATGAGC	TTTACCGCAG
16750	16760	16770	16780	16790	16800
CTGCCTCGCG	CGTTTCGGTG	ATGACGGTGA	AAACCTCTGA	CACATGCAGC	TCCCGGAGAC
16810	16820	16830	16840	16850	16860
GGTCACAGCT	TGTCTGTAAG	CGGATGCCGG	GAGCAGACAA	GCCCCTCAGG	GCGCGTCAGC
16870	16880	16890	16900	16910	16920
GGGTGTTGGC	GGGTGTCGGG	GCGCAGCCAT	GACCCAGTCA	CGTAGCGATA	GCGGAGTGTA

DNAST  
Mandy SE8N-SHL

16930 16940 16950 16960 16970 16980  
 TACTGGCTTA ACTATGCGGC ATCAGAGCAG ATTGTACTGA GAGTGACCA TATGCGGTGT  
 16990 17000 17010 17020 17030 17040  
 GAAATACCGC ACAGATGCGT AAGGAGAAAA TACCGCATCA GCGCTCTTC CGCTTCCTCG  
 17050 17060 17070 17080 17090 17100  
 CTCCTGACT CGCTGCGCTC GGTCGTTCCG CTGCGGCGAG CCGTATCAGC TCACTCAAAG  
 17110 17120 17130 17140 17150 17160  
 GCGGTAATAC GGTTATCCAC AGAATCAGGG GATAACGCAG GAAAGAACAT GTGAGCAAAA  
 17170 17180 17190 17200 17210 17220  
 GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT CCATAGGCTC  
 17230 17240 17250 17260 17270 17280  
 CGCCCCCTG ACGAGCATCA CAAAAATCGA CGCTCAAAGT AGAGGTGGCG AAACCCGACA  
 17290 17300 17310 17320 17330 17340  
 GGACTATAAA GATACCAGGC GTTTCCTCCCT GGAAGCTCCC TCGTGCGCTC TCCTGTTCCG  
 17350 17360 17370 17380 17390 17400  
 ACCCTGCCGC TTACCGGATA CCTGTCCGCC TTTCTCCCTT CGGGAAGCGT GGCGCTTTCT  
 17410 17420 17430 17440 17450 17460  
 CATAGCTCAC GCTGTAGGTA TCTCAGTTCC GTGTAGGTCG TTCGCTCAA GCTGGGCTGT  
 17470 17480 17490 17500 17510 17520  
 GTGCACGAAC CCCCCGTTCA GCGCGACCGC TCGCCCTTAT CCGGTAATA TCGTCTTGAG  
 17530 17540 17550 17560 17570 17580  
 TCCAACCCGG TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC  
 17590 17600 17610 17620 17630 17640  
 AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAAT GGTGGCCTAA CTACGGCTAC  
 17650 17660 17670 17680 17690 17700  
 ACTAGAAGGA CAGTATTTGG TATCTGCGCT CTGCTGAAGC CAGTTACCTT CGGAAAAAGA  
 17710 17720 17730 17740 17750 17760  
 GTTGGTAGCT CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTTT TTTTGTGTC  
 17770 17780 17790 17800 17810 17820  
 AAGCAGCAGA TTACGCGCAG AAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTCTACG  
 17830 17840 17850 17860 17870 17880  
 GGGTCTGACG CTCAGTGGA CGAAAACTCA CGTTAAGGGA TTTTGGTCAT GAGATTATCA  
 17890 17900 17910 17920 17930 17940  
 AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA GTTTTAAATC AATCTAAAGT  
 17950 17960 17970 17980 17990 18000  
 ATATATGAGT AAACCTGGTC TGACAGTTAC CAATGCTTAA TCACTGAGGC ACCTATCTCA  
 18010 18020 18030 18040 18050 18060  
 GCGATCTGTC TATTTCTGTC ATCCATAGTT GCCTGACTCC CCGTCGTGTA GATAACTACG  
 18070 18080 18090 18100 18110 18120  
 ATACGGGAGG GCTTACCATC TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA  
 18130 18140 18150 18160 18170 18180  
 CCGGCTCCAG ATTTATCAGC AATAAACAG CCAGCCGGAA GGGCCGAGCG CAGAAAGTGGT  
 18190 18200 18210 18220 18230 18240

DNASIS

Mandy SE8N-SHL

CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC TAGAGTAAGT

18250	18260	18270	18280	18290	18300
AGTTCGCCAG	TTAATAGTTT	GCGCAACGTT	GTTGCCATTG	CTGCAGGCAT	CGTGGTGTC

18310	18320	18330	18340	18350	18360
CGCTCGTCGT	TTGGTATGGC	TTCAATCAGC	TCCGGTTCCC	AACGATCAAG	GCGAGTTACA

18370	18380	18390	18400	18410	18420
TGATCCCCCA	TGTTGTGCAA	AAAAGCGGTT	AGCTCCTTCG	GTCCTCCGAT	CGTTGTGAGA

18430	18440	18450	18460	18470	18480
AGTAAGTTGG	CCGCAGTGTT	ATCACTCATG	GTTATGGCAG	CACTGCATAA	TTCTCTTACT

18490	18500	18510	18520	18530	18540
GTCATGCCAT	CCGTAAGATG	CTTTTCTGTG	ACTGGTGAGT	ACTCAACCAA	GTCATTCTGA

18550	18560	18570	18580	18590	18600
GAATAGTGTA	TGCGGCGACC	GAGTTGCTCT	TGCCCGGCGT	CAACACGGGA	TAATACCGCG

18610	18620	18630	18640	18650	18660
LCACATAGCA	GAACCTTTAAA	AGTGCTCATC	ATTGGAAGAA	GTTCTTCGGG	GCGAAAACCT

18670	18680	18690	18700	18710	18720
TCAAGGATCT	TACCGCTGTT	GAGATCCAGT	TCGATGTAAC	CCACTCGTGC	ACCCAACCTGA

18730	18740	18750	18760	18770	18780
TCTTCAGCAT	CTTTTACTTT	CACCAGCGTT	TCTGGGTGAG	CAAAAACAGG	AAGGCAAAAT

18790	18800	18810	18820	18830	18840
GCCGCAAAAA	AGGGAATAAG	GCGGACACGG	AAATGTTGAA	TACTCATACT	CTTCTTTTTT

18850	18860	18870	18880	18890	18900
CAATATTATT	GAAGCATTTA	TCAGGGTTAT	TGTCTCATGA	GCGGATACAT	ATTTGAATGT

18910	18920	18930	18940	18950	18960
ATTTAGAAAA	ATAAACAAAT	AGGGGTTCCG	CGCACATTTT	CCCGAAAAGT	GCCACCTGAC

18970	18980	18990	19000	19010	19020
GTCTAAGAAA	CCATTATTAT	CATGACATTA	ACCTATAAAA	ATAGGCGTAT	CACGAGGCCC

19030	19040	19050	19060	19070	19080
TTTCGTCTTC	AAGAA.....	.....	.....	.....	.....

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/03935

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/90 C12N15/85 C12Q1/68 C12N5/10 C12N9/12  
 C12N15/13 C07K16/28 C12N15/12 C07K14/705 G01N33/53  
 C12N15/62 C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 11523 A (IDEC PHARMACEUTICALS CORPORATION (US); REFF MITCHELL E. (US)) 26 May 1994 cited in the application see abstract see page 9, line 21 - page 10, line 29 see page 41, line 19 - page 42, line 19; figure 6	1, 4-8, 11, 12, 25-29, 31, 32
A	US 5 464 764 A (CAPECCHI MARIO R. AND KIRK THOMAS R.) 7 November 1995 see abstract see column 13, line 32 - column 14, line 5	1
A	WO 94 05784 A (UNITED STATES AMERICA REPRESENTED BY THE SECRETARY US DPT. AGRICULTURE) 17 March 1994 see abstract	1

-/--

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

23 July 1998

Date of mailing of the international search report

05/08/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Macchia, G

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/03935

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 24642 A (TSI CORPORATION (US)) 9 December 1993 see abstract	1
A	----- BARNETT R.S. ET AL.: "Antibody production in chinese hamster ovary cells using an impaired selectable marker" ACS SYMPOSIUM SERIES: ANTIBODY EXPRESSION AND ENGINEERING, vol. 604, 1995, pages 27-40, XP002072464 -----	

# INTERNATIONAL SEARCH REPORT

I. .ational Application No

PCT/US 98/03935

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9411523 A	26-05-1994	AU 682481 B	09-10-1997
		AU 5613294 A	08-06-1994
		CA 2149326 A	26-05-1994
		DE 669986 T	10-10-1996
		EP 0669986 A	06-09-1995
		ES 2088838 T	01-10-1996
		JP 8503138 T	09-04-1996
		US 5648267 A	15-07-1997
		US 5733779 A	31-03-1998
US 5464764 A	07-11-1995	US 5487992 A	30-01-1996
		US 5627059 A	06-05-1997
		US 5631153 A	20-05-1997
WO 9405784 A	17-03-1994	AU 4839493 A	29-03-1994
		MX 9305183 A	31-05-1994
WO 9324642 A	09-12-1993	AU 4401993 A	30-12-1993



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup>:</b> <b>C12N 15/90, 15/85, C12Q 1/68, C12N 5/10, 9/12, 15/13, C07K 16/28, C12N 15/12, C07K 14/705, G01N 33/53, C12N 15/62, C07K 19/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/41645</b>  <b>(43) International Publication Date:</b> 24 September 1998 (24.09.98)
<b>(21) International Application Number:</b> PCT/US98/03935  <b>(22) International Filing Date:</b> 9 March 1998 (09.03.98)  <b>(30) Priority Data:</b> 08/819,866           14 March 1997 (14.03.97)       US 09/023,715           13 February 1998 (13.02.98)      US  <b>(71) Applicant:</b> IDEC PHARMACEUTICALS CORPORATION [US/US]; 11011 Torreyana Road, San Diego, CA 92121 (US).  <b>(72) Inventors:</b> REFF, Mitchell, E.; 4166 Combe Way, San Diego, CA 92122 (US). BARNETT, Richard, Spence; 306 Belmont Court, San Marcos, CA 92069 (US). McLACHLAN, Karen, Retta; Apartment B6, 766 South Nardo, Solana Beach, CA 92075 (US).  <b>(74) Agents:</b> GESS, E., Joseph et al.; Burns, Doane, Swecker & Mathis L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(54) Title:</b> METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME		
<b>(57) Abstract</b>  A method for achieving site specific integration of a desired DNA at a target site in a mammalian cell via homologous recombination is described. This method provides for the reproducible selection of cell lines wherein a desired DNA is integrated at a predetermined transcriptionally active site previously marked with a marker plasmid. The method is particularly suitable for the production of mammalian cell lines which secrete mammalian proteins at high levels, in particular immunoglobulins. Vectors and vector combinations for use in the subject cloning method are also provided.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

### Title of the Invention

METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA  
HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME

5

### Field of the Invention

The present invention relates to a process of targeting the integration of a desired exogenous DNA to a specific location within the genome of a mammalian cell.

10 More specifically, the invention describes a novel method for identifying a transcriptionally active target site ("hot spot") in the mammalian genome, and inserting a desired DNA at this site via homologous recombination. The invention also optionally provides the ability for

15 gene amplification of the desired DNA at this location by co-integrating an amplifiable selectable marker, e.g., DHFR, in combination with the exogenous DNA. The invention additionally describes the construction of novel vectors suitable for accomplishing the above, and

20 further provides mammalian cell lines produced by such methods which contain a desired exogenous DNA integrated at a target hot spot.

- 2 -

Background

Technology for expressing recombinant proteins in both prokaryotic and eukaryotic organisms is well established. Mammalian cells offer significant advantages over bacteria or yeast for protein production, resulting from their ability to correctly assemble, glycosylate and post-translationally modify recombinantly expressed proteins. After transfection into the host cells, recombinant expression constructs can be maintained as extrachromosomal elements, or may be integrated into the host cell genome. Generation of stably transfected mammalian cell lines usually involves the latter; a DNA construct encoding a gene of interest along with a drug resistance gene (dominant selectable marker) is introduced into the host cell, and subsequent growth in the presence of the drug allows for the selection of cells that have successfully integrated the exogenous DNA. In many instances, the gene of interest is linked to a drug resistant selectable marker which can later be subjected to gene amplification. The gene encoding dihydrofolate reductase (DHFR) is most commonly used for this purpose. Growth of cells in the presence of methotrexate, a competitive inhibitor of DHFR, leads to increased DHFR production by means of amplification of the DHFR gene. As flanking regions of DNA will also become amplified, the resultant coamplification of a DHFR linked gene in the transfected cell line can lead to increased protein

- 3 -

production, thereby resulting in high level expression of the gene of interest.

While this approach has proven successful, there are a number of problems with the system because of the random nature of the integration event. These problems exist because expression levels are greatly influenced by the effects of the local genetic environment at the gene locus, a phenomena well documented in the literature and generally referred to as "position effects" (for example, see Al-Shawi et al, *Mol. Cell. Biol.*, 10:1192-1198 (1990); Yoshimura et al, *Mol. Cell. Biol.*, 7:1296-1299 (1987)). As the vast majority of mammalian DNA is in a transcriptionally inactive state, random integration methods offer no control over the transcriptional fate of the integrated DNA. Consequently, wide variations in the expression level of integrated genes can occur, depending on the site of integration. For example, integration of exogenous DNA into inactive, or transcriptionally "silent" regions of the genome will result in little or no expression. By contrast integration into a transcriptionally active site may result in high expression.

Therefore, when the goal of the work is to obtain a high level of gene expression, as is typically the desired outcome of genetic engineering methods, it is generally necessary to screen large numbers of transfectants to find such a high producing clone.

- 4 -

Additionally, random integration of exogenous DNA into the genome can in some instances disrupt important cellular genes, resulting in an altered phenotype. These factors can make the generation of high expressing stable mammalian cell lines a complicated and laborious process.

Recently, our laboratory has described the use of DNA vectors containing translationally impaired dominant selectable markers in mammalian gene expression. (This is disclosed in U.S. Serial No. 08/147,696 filed November 3, 1993, recently allowed).

These vectors contain a translationally impaired neomycin phosphotransferase (neo) gene as the dominant selectable marker, artificially engineered to contain an intron into which a DHFR gene along with a gene or genes of interest is inserted. Use of these vectors as expression constructs has been found to significantly reduce the total number of drug resistant colonies produced, thereby facilitating the screening procedure in relation to conventional mammalian expression vectors. Furthermore, a significant percentage of the clones obtained using this system are high expressing clones. These results are apparently attributable to the modifications made to the neo selectable marker. Due to the translational impairment of the neo gene, transfected cells will not produce enough neo protein to survive drug selection, thereby decreasing the overall

- 5 -

number of drug resistant colonies. Additionally, a higher percentage of the surviving clones will contain the expression vector integrated into sites in the genome where basal transcription levels are high, resulting in overproduction of neo, thereby allowing the cells to overcome the impairment of the neo gene. Concomitantly, the genes of interest linked to neo will be subject to similar elevated levels of transcription. This same advantage is also true as a result of the artificial intron created within neo; survival is dependent on the synthesis of a functional neo gene, which is in turn dependent on correct and efficient splicing of the neo introns. Moreover, these criteria are more likely to be met if the vector DNA has integrated into a region which is already highly transcriptionally active.

Following integration of the vector into a transcriptionally active region, gene amplification is performed by selection for the DHFR gene. Using this system, it has been possible to obtain clones selected using low levels of methotrexate (50nM), containing few (<10) copies of the vector which secrete high levels of protein (>55pg/cell/day). Furthermore, this can be achieved in a relatively short period of time. However, the success in amplification is variable. Some transcriptionally active sites cannot be amplified and

- 6 -

therefore the frequency and extent of amplification from a particular site is not predictable.

Overall, the use of these translationally impaired vectors represents a significant improvement over other methods of random integration. However, as discussed, the problem of lack of control over the integration site remains a significant concern.

One approach to overcome the problems of random integration is by means of gene targeting, whereby the exogenous DNA is directed to a specific locus within the host genome. The exogenous DNA is inserted by means of homologous recombination occurring between sequences of DNA in the expression vector and the corresponding homologous sequence in the genome. However, while this type of recombination occurs at a high frequency naturally in yeast and other fungal organisms, in higher eukaryotic organisms it is an extremely rare event. In mammalian cells, the frequency of homologous versus non-homologous (random integration) recombination is reported to range from 1/100 to 1/5000 (for example, see Capecchi, *Science*, 244:1288-1292 (1989); Morrow and Kucherlapati, *Curr. Op. Biotech.*, 4:577-582 (1993)).

One of the earliest reports describing homologous recombination in mammalian cells comprised an artificial system created in mouse fibroblasts (Thomas et al, *Cell*, 44:419-428 (1986)). A cell line containing a mutated, non-functional version of the neo gene integrated into

- 7 -

the host genome was created, and subsequently targeted with a second non-functional copy of neo containing a different mutation. Reconstruction of a functional neo gene could occur only by gene targeting. Homologous recombina-  
5 recombinants were identified by selecting for G418 resistant cells, and confirmed by analysis of genomic DNA isolated from the resistant clones.

Recently, the use of homologous recombination to replace the heavy and light immunoglobulin genes at  
10 endogenous loci in antibody secreting cells has been reported. (U.S. Patent No. 5,202,238, Fell et al, (1993).) However, this particular approach is not widely applicable, because it is limited to the production of immunoglobulins in cells which  
15 endogenously express immunoglobulins, e.g., B cells and myeloma cells. Also, expression is limited to single copy gene levels because co-amplification after homologous recombination is not included. The method is further complicated by the fact that two separate  
20 integration events are required to produce a functional immunoglobulin: one for the light chain gene followed by one for the heavy chain gene.

An additional example of this type of system has been reported in NS/O cells, where recombinant  
25 immunoglobulins are expressed by homologous recombination into the immunoglobulin gamma 2A locus (Hollis et al, international patent application #

- 8 -

PCT/IB95 (00014).) Expression levels obtained from this site were extremely high - on the order of 20pg/cell/day from a single copy integrant. However, as in the above example, expression is limited to this level because an amplifiable gene is not contegrated in this system. Also, other researchers have reported aberrant glycosylation of recombinant proteins expressed in NS/O cells (for example, see Flesher et al, *Biotech. and Bioeng.*, 48:399-407 (1995)), thereby limiting the applicability of this approach.

The cre-loxP recombination system from bacteriophage P1 has recently been adapted and used as a means of gene targeting in eukaryotic cells. Specifically, the site specific integration of exogenous DNA into the Chinese hamster ovary (CHO) cell genome using cre recombinase and a series of lox containing vectors have been described. (Fukushige and Sauer, *Proc. Natl. Acad. Sci. USA*, 89:7905-7909 (1992).) This system is attractive in that it provides for reproducible expression at the same chromosomal location. However, no effort was made to identify a chromosomal site from which gene expression is optimal, and as in the above example, expression is limited to single copy levels in this system. Also, it is complicated by the fact that one needs to provide for expression of a functional recombinase enzyme in the mammalian cell.

- 9 -

The use of homologous recombination between an introduced DNA sequence and its endogenous chromosomal locus has also been reported to provide a useful means of genetic manipulation in mammalian cells, as well as in yeast cells. (See e.g., Bradley et al, *Meth. Enzymol.*, 223:855-879 (1993); Capecchi, *Science*, 244:1288-1292 (1989); Rothstein et al, *Meth. Enzymol.*, 194:281-301 (1991)). To date, most mammalian gene targeting studies have been directed toward gene disruption ("knockout") or site-specific mutagenesis of selected target gene loci in mouse embryonic stem (ES) cells. The creation of these "knockout" mouse models has enabled scientists to examine specific structure-function issues and examine the biological importance of a myriad of mouse genes. This field of research also has important implications in terms of potential gene therapy applications.

Also, vectors have recently been reported by Celltech (Kent, U.K.) which purportedly are targeted to transcriptionally active sites in NSO cells, which do not require gene amplification (Peakman et al, *Hum. Antibod. Hybridomas*, 5:65-74 (1994)). However, levels of immunoglobulin secretion in these unamplified cells have not been reported to exceed 20pg/cell/day, while in amplified CHO cells, levels as high as 100pg/cell/day can be obtained (*Id.*).

- 10 -

It would be highly desirable to develop a gene targeting system which reproducibly provided for the integration of exogenous DNA into a predetermined site in the genome known to be transcriptionally active.

5 Also, it would be desirable if such a gene targeting system would further facilitate co-amplification of the inserted DNA after integration. The design of such a system would allow for the reproducible and high level expression of any cloned gene of interest in a mammalian  
10 cell, and undoubtedly would be of significant interest to many researchers.

In this application, we provide a novel mammalian expression system, based on homologous recombination occurring between two artificial substrates contained in  
15 two different vectors. Specifically, this system uses a combination of two novel mammalian expression vectors, referred to as a "marking" vector and a "targeting" vector.

Essentially, the marking vector enables the identification and marking of a site in the mammalian genome  
20 which is transcriptionally active, i.e., a site at which gene expression levels are high. This site can be regarded as a "hot spot" in the genome. After integration of the marking vector, the subject expression system enables another DNA to be integrated at this site,  
25 i.e., the targeting vector, by means of homologous recombination occurring between DNA sequences common to

- 11 -

both vectors. This system affords significant advantages over other homologous recombination systems.

Unlike most other homologous systems employed in mammalian cells, this system exhibits no background.

5 Therefore, cells which have only undergone random integration of the vector do not survive the selection.

Thus, any gene of interest cloned into the targeting plasmid is expressed at high levels from the marked hot spot. Accordingly, the subject method of gene expres-  
10 sion substantially or completely eliminates the problems

inherent to systems of random integration, discussed in detail above. Moreover, this system provides reproducible and high level expression of any recombinant protein at the same transcriptionally active site in the  
15 mammalian genome. In addition, gene amplification may be effected at this particular transcriptionally active site by including an amplifiable dominant selectable marker (e.g. DHFR) as part of the marking vector.

#### Objects of the Invention

20 Thus, it is an object of the invention to provide an improved method for targeting a desired DNA to a specific site in a mammalian cell.

It is a more specific object of the invention to provide a novel method for targeting a desired DNA to a  
25 specific site in a mammalian cell via homologous recombination.

- 12 -

It is another specific object of the invention to provide novel vectors for achieving site specific integration of a desired DNA in a mammalian cell.

5 It is still another object of the invention to provide novel mammalian cell lines which contain a desired DNA integrated at a predetermined site which provides for high expression.

10 It is a more specific object of the invention to provide a novel method for achieving site specific integration of a desired DNA in a Chinese hamster ovary (CHO) cell.

15 It is another more specific object of the invention to provide a novel method for integrating immunoglobulin genes, or any other genes, in mammalian cells at predetermined chromosomal sites that provide for high expression.

20 It is another specific object of the invention to provide novel vectors and vector combinations suitable for integrating immunoglobulin genes into mammalian cells at predetermined sites that provide for high expression.

25 It is another object of the invention to provide mammalian cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high expression.

It is an even more specific object of the invention to provide a novel method for integrating immunoglobulin

- 13 -

genes into CHO cells that provide for high expression, as well as novel vectors and vector combinations that provide for such integration of immunoglobulin genes into CHO cells.

5 In addition, it is a specific object of the invention to provide novel CHO cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high expression, and have been amplified by methotrexate selection to secrete even greater amounts  
10 of functional immunoglobulins.

#### Brief Description of the Figures

Figure 1 depicts a map of a marking plasmid according to the invention referred to as Desmond. The plasmid is shown in circular form (1a) as well as a  
15 linearized version used for transfection (1b).

Figure 2(a) shows a map of a targeting plasmid referred to "Molly". Molly is shown here encoding the anti-CD20 immunoglobulin genes, expression of which is described in Example 1.

20 Figure 2(b) shows a linearized version of Molly, after digestion with the restriction enzymes KpnI and PacI. This linearized form was used for transfection.

Figure 3 depicts the potential alignment between Desmond sequences integrated into the CHO genome, and  
25 incoming targeting Molly sequences. One potential ar-

- 14 -

rangement of Molly integrated into Desmond after homologous recombination is also presented.

Figure 4 shows a Southern analysis of single copy Desmond clones. Samples are as follows:

- 5 Lane 1:  $\lambda$ HindIII DNA size marker
- Lane 2: Desmond clone 10F3
- Lane 3: Desmond clone 10C12
- Lane 4: Desmond clone 15C9
- Lane 5: Desmond clone 14B5
- 10 Lane 6: Desmond clone 9B2

Figure 5 shows a Northern analysis of single copy Desmond clones. Samples are as follows: Panel A: northern probed with CAD and DHFR probes, as indicated on the figure. Panel B: duplicate northern, probed with CAD and HisD probes, as indicated. The RNA samples loaded in panels A and B are as follows:

- 15 Lane 1: clone 9B2, lane 2; clone 10C12, lane 3; clone 14B5, lane 4; clone 15C9, lane 5; control RNA from CHO transfected with a HisD and DHFR containing plasmid,
- 20 lane 6; untransfected CHO.

Figure 6 shows a Southern analysis of clones resulting from the homologous integration of Molly into Desmond. Samples are as follows:

- Lane 1:  $\lambda$ HindIII DNA size markers, Lane 2: 20F4, lane 3;
- 25 5F9, lane 4; 21C7, lane 5; 24G2, lane 6; 25E1, lane 7; 28C9, lane 8; 29F9, lane 9; 39G11, lane 10; 42F9, lane 11; 50G10, lane 12; Molly plasmid DNA, linearized with

- 15 -

BglIII (top band) and cut with BglIII and KpnI (lower band), lane 13; untransfected Desmond.

Figures 7A through 7G contain the Sequence Listing for Desmond.

5        Figures 8A through 8I contain the Sequence Listing for Molly-containing anti-CD20.

Figure 9 contains a map of the targeting plasmid, "Mandy," shown here encoding anti-CD23 genes, the expression of which is disclosed in Example 5.

10       Figures 10A through 10N contain the sequence listing of "Mandy" containing the anti-CD23 genes as disclosed in Example 5.

#### Detailed Description of the Invention

15       The invention provides a novel method for integrating a desired exogenous DNA at a target site within the genome of a mammalian cell via homologous recombination. Also, the invention provides novel vectors for achieving the site specific integration of a DNA at a target site in the genome of a mammalian cell.

20       More specifically, the subject cloning method provides for site specific integration of a desired DNA in a mammalian cell by transfection of such cell with a "marker plasmid" which contains a unique sequence that is foreign to the mammalian cell genome and which  
25       provides a substrate for homologous recombination, followed by transfection with a "target plasmid" containing

- 16 -

a sequence which provides for homologous recombination with the unique sequence contained in the marker plasmid, and further comprising a desired DNA that is to be integrated into the mammalian cell. Typically, the integrated DNA will encode a protein of interest, such as an immunoglobulin or other secreted mammalian glycoprotein.

The exemplified homologous recombination system uses the neomycin phosphotransferase gene as a dominant selectable marker. This particular marker was utilized based on the following previously published observations;

(i) the demonstrated ability to target and restore function to a mutated version of the neo gene (cited earlier) and

(ii) our development of translationally impaired expression vectors, in which the neo gene has been artificially created as two exons with a gene of interest inserted in the intervening intron; neo exons are correctly spliced and translated in vivo, producing a functional protein and thereby conferring G418 resistance on the resultant cell population. In this application, the neo gene is split into three exons. The third exon of neo is present on the "marker" plasmid and becomes integrated into the host cell genome upon integration of the marker plasmid into the mammalian cells. Exons 1 and 2 are present on the targeting plasmid, and are separated

- 17 -

by an intervening intron into which at least one gene of interest is cloned. Homologous recombination of the targeting vector with the integrated marking vector results in correct splicing of all three exons of the neo gene and thereby expression of a functional neo protein (as determined by selection for G418 resistant colonies). Prior to designing the current expression system, we had experimentally tested the functionality of such a triply spliced neo construct in mammalian cells. The results of this control experiment indicated that all three neo exons were properly spliced and therefore suggested the feasibility of the subject invention.

However, while the present invention is exemplified using the neo gene, and more specifically a triple split neo gene, the general methodology should be efficacious with other dominant selectable markers.

As discussed in greater detail *infra*, the present invention affords numerous advantages to conventional gene expression methods, including both random integration and gene targeting methods. Specifically, the subject invention provides a method which reproducibly allows for site-specific integration of a desired DNA into a transcriptionally active domain of a mammalian cell. Moreover, because the subject method introduces an artificial region of "homology" which acts as a unique substrate for homologous recombination and the

- 18 -

insertion of a desired DNA, the efficacy of subject invention does not require that the cell endogenously contain or express a specific DNA. Thus, the method is generically applicable to all mammalian cells, and can  
5 be used to express any type of recombinant protein.

The use of a triply spliced selectable marker, e.g., the exemplified triply spliced neo construct, guarantees that all G418 resistant colonies produced will arise from a homologous recombination event (random  
10 integrants will not produce a functional neo gene and consequently will not survive G418 selection). Thus, the subject invention makes it easy to screen for the desired homologous event. Furthermore, the frequency of additional random integrations in a cell that has under-  
15 gone a homologous recombination event appears to be low.

Based on the foregoing, it is apparent that a significant advantage of the invention is that it substantially reduces the number of colonies that need be screened to identify high producer clones, i.e., cell  
20 lines containing a desired DNA which secrete the corresponding protein at high levels. On average, clones containing integrated desired DNA may be identified by screening about 5 to 20 colonies (compared to several thousand which must be screened when using standard  
25 random integration techniques, or several hundred using the previously described intronic insertion vectors) Additionally, as the site of integration was preselected

- 19 -

and comprises a transcriptionally active domain, all exogenous DNA expressed at this site should produce comparable, i.e. high levels of the protein of interest.

Moreover, the subject invention is further advantageous in that it enables an amplifiable gene to be inserted on integration of the marking vector. Thus, when a desired gene is targeted to this site via homologous recombination, the subject invention allows for expression of the gene to be further enhanced by gene amplification. In this regard, it has been reported in from the literature that different genomic sites have different capacities for gene amplification (Meinkoth et al, *Mol. Cell Biol.*, 7:1415-1424 (1987)). Therefore, this technique is further advantageous as it allows for the placement of a desired gene of interest at a specific site that is both transcriptionally active and easily amplified. Therefore, this should significantly reduce the amount of time required to isolate such high producers.

Specifically, while conventional methods for the construction of high expressing mammalian cell lines can take 6 to 9 months, the present invention allows for such clones to be isolated on average after only about 3-6 months. This is due to the fact that conventionally isolated clones typically must be subjected to at least three rounds of drug resistant gene amplification in order to reach satisfactory levels of gene expression.

- 20 -

As the homologously produced clones are generated from a preselected site which is a high expression site, fewer rounds of amplification should be required before reaching a satisfactory level of production.

5        Still further, the subject invention enables the reproducible selection of high producer clones wherein the vector is integrated at low copy number, typically single copy. This is advantageous as it enhances the stability of the clones and avoids other potential adverse side-effects associated with high copy number. As  
10        described *supra*, the subject homologous recombination system uses the combination of a "marker plasmid" and a "targeting plasmid" which are described in more detail below.

15        The "marker plasmid" which is used to mark and identify a transcriptionally hot spot will comprise at least the following sequences:

(i) a region of DNA that is heterologous or unique to the genome of the mammalian cell, which functions as  
20        a source of homology, allows for homologous recombination (with a DNA contained in a second target plasmid). More specifically, the unique region of DNA (i) will generally comprise a bacterial, viral, yeast synthetic, or other DNA which is not normally present in the  
25        mammalian cell genome and which further does not comprise significant homology or sequence identity to DNA contained in the genome of the mammalian cell.

- 21 -

Essentially, this sequence should be sufficiently different to mammalian DNA that it will not significantly recombine with the host cell genome via homologous recombination. The size of such unique DNA will generally be at least about 2 to 10 kilobases in size, or higher, more preferably at least about 10kb, as several other investigators have noted an increased frequency of targeted recombination as the size of the homology region is increased (Capecchi, *Science*, 244:1288-1292 (1989)).

The upper size limit of the unique DNA which acts as a site for homologous recombination with a sequence in the second target vector is largely dictated by potential stability constraints (if DNA is too large it may not be easily integrated into a chromosome and the difficulties in working with very large DNAs.

(ii) a DNA including a fragment of a selectable marker DNA, typically an exon of a dominant selectable marker gene. The only essential feature of this DNA is that it not encode a functional selectable marker protein unless it is expressed in association with a sequence contained in the target plasmid. Typically, the target plasmid will comprise the remaining exons of the dominant selectable marker gene (those not comprised in "targeting" plasmid). Essentially, a functional selectable marker should only be produced if homologous recombination occurs (resulting in the association and

- 22 -

expression of this marker DNA (i) sequence together with the portion(s) of the selectable marker DNA fragment which is (are) contained in the target plasmid).

As noted, the current invention exemplifies the use of the neomycin phosphotransferase gene as the dominant selectable marker which is "split" in the two vectors. However, other selectable markers should also be suitable, e.g., the Salmonella histidinol dehydrogenase gene, hygromycin phosphotransferase gene, herpes simplex virus thymidine kinase gene, adenosine deaminase gene, glutamine synthetase gene and hypoxanthine-guanine phosphoribosyl transferase gene.

(iii) a DNA which encodes a functional selectable marker protein, which selectable marker is different from the selectable marker DNA (ii). This selectable marker provides for the successful selection of mammalian cells wherein the marker plasmid is successfully integrated into the cellular DNA. More preferably, it is desirable that the marker plasmid comprise two such dominant selectable marker DNAs, situated at opposite ends of the vector. This is advantageous as it enables integrants to be selected using different selection agents and further enables cells which contain the entire vector to be selected. Additionally, one marker can be an amplifiable marker to facilitate gene amplification as discussed previously. Any of the

- 23 -

dominant selectable marker listed in (ii) can be used as well as others generally known in the art.

Moreover, the marker plasmid may optionally further comprise a rare endonuclease restriction site. This is potentially desirable as this may facilitate cleavage.

If present, such rare restriction site should be situated close to the middle of the unique region that acts as a substrate for homologous recombination. Preferably such sequence will be at least about 12 nucleotides.

The introduction of a double stranded break by similar methodology has been reported to enhance the frequency of homologous recombination. (Choulika et al, *Mol. Cell. Biol.*, 15:1968-1973 (1995)). However, the presence of such sequence is not essential.

The "targeting plasmid" will comprise at least the following sequences:

(1) the same unique region of DNA contained in the marker plasmid or one having sufficient homology or sequence identity therewith that said DNA is capable of combining via homologous recombination with the unique region (i) in the marker plasmid. Suitable types of DNAs are described *supra* in the description of the unique region of DNA (1) in the marker plasmid.

(2) The remaining exons of the dominant selectable marker, one exon of which is included as (ii) in the marker plasmid listed above. The essential features of this DNA fragment is that it result in a functional

- 24 -

(selectable) marker protein only if the target plasmid integrates via homologous recombination (wherein such recombination results in the association of this DNA with the other fragment of the selectable marker DNA contained in the marker plasmid) and further that it allow for insertion of a desired exogenous DNA. Typically, this DNA will comprise the remaining exons of the selectable marker DNA which are separated by an intron. For example, this DNA may comprise the first two exons of the neo gene and the marker plasmid may comprise the third exon (back third of neo).

(3) The target plasmid will also comprise a desired DNA, e.g., one encoding a desired polypeptide, preferably inserted within the selectable marker DNA fragment contained in the plasmid. Typically, the DNA will be inserted in an intron which is comprised between the exons of the selectable marker DNA. This ensures that the desired DNA is also integrated if homologous recombination of the target plasmid and the marker plasmid occurs. This intron may be naturally occurring or it may be engineered into the dominant selectable marker DNA fragment.

This DNA will encode any desired protein, preferably one having pharmaceutical or other desirable properties. Most typically the DNA will encode a mammalian protein, and in the current examples provided, an immunoglobulin or an immunoadhesin. However the

- 25 -

invention is not in any way limited to the production of immunoglobulins.

As discussed previously, the subject cloning method is suitable for any mammalian cell as it does not require for efficacy that any specific mammalian sequence or sequences be present. In general, such mammalian cells will comprise those typically used for protein expression, e.g., CHO cells, myeloma cells, COS cells, BHK cells, Sp2/0 cells, NIH 3T3 and HeLa cells. In the examples which follow, CHO cells were utilized. The advantages thereof include the availability of suitable growth medium, their ability to grow efficiently and to high density in culture, and their ability to express mammalian proteins such as immunoglobulins in biologically active form.

Further, CHO cells were selected in large part because of previous usage of such cells by the inventors for the expression of immunoglobulins (using the translationally impaired dominant selectable marker containing vectors described previously). Thus, the present laboratory has considerable experience in using such cells for expression. However, based on the examples which follow, it is reasonable to expect similar results will be obtained with other mammalian cells.

In general, transformation or transfection of mammalian cells according to the subject invention will be effected according to conventional methods. So that the

- 26 -

invention may be better understood, the construction of exemplary vectors and their usage in producing integrants is described in the examples below.

#### EXAMPLE 1

5

##### Design and Preparation of Marker and Targeting Plasmid DNA Vectors

The marker plasmid herein referred to as "Desmond" was assembled from the following DNA elements:

10 (a) Murine dihydrofolate reductase gene (DHFR), incorporated into a transcription cassette, comprising the mouse beta globin promoter 5" to the DHFR start site, and bovine growth hormone poly adenylation signal 3" to the stop codon. The DHFR transcriptional cassette was isolated from TCAE6, an expression vector created  
15 previously in this laboratory (Newman et al, 1992, *Bio-technology*, 10:1455-1460).

(b) E. coli  $\beta$ -galactosidase gene - commercially available, obtained from Promega as pSV-b-galactosidase control vector, catalog # E1081.

20 (c) Baculovirus DNA, commercially available, purchased from Clontech as pBAKPAK8, cat # 6145-1.

(d) Cassette comprising promoter and enhancer elements from Cytomegalovirus and SV40 virus. The cassette was generated by PCR using a derivative of expression  
25 vector TCAE8 (Reff et al, *Blood*, 83:435-445 (1994)). The enhancer cassette was inserted within the baculo-

- 27 -

virus sequence, which was first modified by the insertion of a multiple cloning site.

(e) E. coli GUS (glucuronidase) gene, commercially available, purchased from Clontech as pB101, cat. #  
5 6017-1.

(f) Firefly luciferase gene, commercially available, obtained from Promega as pGEM-Luc (catalog # E1541).

(g) S. typhimurium histidinol dehydrogenase gene  
10 (HisD). This gene was originally a gift from (Donahue et al, Gene, 18:47-59 (1982)), and has subsequently been incorporated into a transcription cassette comprising the mouse beta globin major promoter 5' to the gene, and the SV40 polyadenylation signal 3' to the gene.

15 The DNA elements described in (a)-(g) were combined into a pBR derived plasmid backbone to produce a 7.7kb contiguous stretch of DNA referred to in the attached figures as "homology". Homology in this sense refers to sequences of DNA which are not part of the mammalian  
20 genome and are used to promote homologous recombination between transfected plasmids sharing the same homology DNA sequences.

(h) Neomycin phosphotransferase gene from TN5 (Davis and Smith, Ann. Rev. Micro., 32:469-518 (1978)).

25 The complete neo gene was subcloned into pBluescript SK-(Stratagene catalog # 212205) to facilitate genetic manipulation. A synthetic linker was then inserted into

- 28 -

a unique Pst1 site occurring across the codons for amino acid 51 and 52 of neo. This linker encoded the necessary DNA elements to create an artificial splice donor site, intervening intron and splice acceptor site within the neo gene, thus creating two separate exons, presently referred to as neo exon 1 and 2. Neo exon 1 encodes the first 51 amino acids of neo, while exon 2 encodes the remaining 203 amino acids plus the stop codon of the protein A Not1 cloning site was also created within the intron.

Neo exon 2 was further subdivided to produce neo exons 2 and 3. This was achieved as follows: A set of PCR primers were designed to amplify a region of DNA encoding neo exon 1, intron and the first 111 2/3 amino acids of exon2. The 3' PCR primer resulted in the introduction of a new 5' splice site immediately after the second nucleotide of the codon for amino acid 111 in exon 2, therefore generating a new smaller exon 2. The DNA fragment now encoding the original exon 1, intron and new exon 2 was then subcloned and propagated in a pBR based vector. The remainder of the original exon 2 was used as a template for another round of PCR amplification, which generated "exon3". The 5' primer for this round of amplification introduced a new splice acceptor site at the 5' side of the newly created exon 3, i.e. before the final nucleotide of the codon for amino acid 111. The resultant 3 exons of neo encode the

- 29 -

following information: exon 1 - the first 51 amino acids of neo; exon 2 - the next 111 2/3 amino acids, and exon 3 the final 91 1/3 amino acids plus the translational stop codon of the neo gene.

5        Neo exon 3 was incorporated along with the above mentioned DNA elements into the marking plasmid "Desmond". Neo exons 1 and 2 were incorporated into the targeting plasmid "Molly". The NotI cloning site created within the intron between exons 1 and 2 was used in  
10 subsequent cloning steps to insert genes of interest into the targeting plasmid.

A second targeting plasmid "Mandy" was also generated. This plasmid is almost identical to "Molly" (some restriction sites on the vector have been changed)  
15 except that the original HisD and DHFR genes contained in "Molly" were inactivated. These changes were incorporated because the Desmond cell line was no longer being cultured in the presence of Histidinol, therefore it seemed unnecessary to include a second copy of the  
20 HisD gene. Additionally, the DHFR gene was inactivated to ensure that only a single DHFR gene, namely the one present in the Desmond marked site, would be amplifiable in any resulting cell lines. "Mandy" was derived from "Molly" by the following modifications:

25        (i) A synthetic linker was inserted in the middle of the DHFR coding region. This linker created a stop codon and shifted the remainder of the DHFR coding

- 30 -

region out of frame, therefore rendering the gene nonfunctional.

(ii) A portion of the HisD gene was deleted and replaced with a PCR generated HisD fragment lacking the promoter and start codon of the gene.

Figure 1 depicts the arrangement of these DNA elements in the marker plasmid "Desmond". Figure 2 depicts the arrangement of these elements in the first targeting plasmid, "Molly". Figure 3 illustrates the possible arrangement in the CHO genome, of the various DNA elements after targeting and integration of Molly DNA into Desmond marked CHO cells. Figure 9 depicts the targeting plasmid "Mandy."

Construction of the marking and targeting plasmids from the above listed DNA elements was carried out following conventional cloning techniques (see, e.g., Molecular Cloning, A Laboratory Manual, J. Sambrook et al, 1987, Cold Spring Harbor Laboratory Press, and Current Protocols in Molecular Biology, F. M. Ausubel et al, eds., 1987, John Wiley and Sons). All plasmids were propagated and maintained in E. coli XLI blue (Stratagene, cat. # 200236). Large scale plasmid preparations were prepared using Promega Wizard Maxiprep DNA Purification System®, according to the manufacturer's directions.

- 31 -

EXAMPLE 2Construction of a Marked CHO Cell Line**1. Cell Culture and Transfection Procedures to Produced Marked CHO Cell Line**

5           Marker plasmid DNA was linearized by digestion overnight at 37°C with Bst1107I. Linearized vector was ethanol precipitated and resuspended in sterile TE to a concentration of 1mg/ml. Linearized vector was introduced into DHFR-Chinese hamster ovary cells (CHO cells) 10 DG44 cells (Urlaub et al, *Som. Cell and Mol. Gen.*, 12:555-566 (1986)) by electroporation as follows.

Exponentially growing cells were harvested by centrifugation, washed once in ice cold SBS (sucrose buffered solution, 272mM sucrose, 7mM sodium phosphate, 15 pH 7.4, 1mM magnesium chloride) then resuspended in SBS to a concentration of  $10^7$  cells/ml. After a 15 minute incubation on ice, 0.4ml of the cell suspension was mixed with 40 $\mu$ g linearized DNA in a disposable electroporation cuvette. Cells were shocked using a BTX 20 electrocell manipulator (San Diego, CA) set at 230 volts, 400 microfaraday capacitance, 13 ohm resistance. Shocked cells were then mixed with 20 ml of prewarmed CHO growth media (CHO-S-SFMII, Gibco/BRL, catalog # 31033-012) and plated in 96 well tissue culture plates. 25 Forty eight hours after electroporation, plates were fed with selection media (in the case of transfection with Desmond, selection media is CHO-S-SFMII without

- 32 -

hypoxanthine or thymidine, supplemented with 2mM  
Histidinol (Sigma catalog # H6647)). Plates were main-  
tained in selection media for up to 30 days, or until  
some of the wells exhibited cell growth. These cells  
5 were then removed from the 96 well plates and expanded  
ultimately to 120 ml spinner flasks where they were  
maintained in selection media at all times.

### EXAMPLE 3

#### Characterization of Marked CHO Cell Lines

##### 10 (a) Southern Analysis

Genomic DNA was isolated from all stably growing  
Desmond marked CHO cells. DNA was isolated using the  
Invitrogen Easy® DNA kit, according to the manufactur-  
er's directions. Genomic DNA was then digested with  
15 HindIII overnight at 37°C, and subjected to Southern  
analysis using a PCR generated digoxigenin labelled  
probe specific to the DHFR gene. Hybridizations and  
washes were carried out using Boehringer Mannheim's DIG  
easy hyb (catalog # 1603 558) and DIG Wash and Block  
20 Buffer Set (catalog # 1585 762) according to the manu-  
facturer's directions. DNA samples containing a single  
band hybridizing to the DHFR probe were assumed to be  
Desmond clones arising from a single cell which had  
integrated a single copy of the plasmid. These clones  
25 were retained for further analysis. Out of a total of  
45 HisD resistant cell lines isolated, only 5 were

- 33 -

single copy integrants. Figure 4 shows a Southern blot containing all 5 of these single copy Desmond clones.

Clone names are provided in the figure legend.

(b) Northern Analysis

5        Total RNA was isolated from all single copy Desmond clones using TRizol reagent (Gibco/BRL cat # 15596-026) according to the manufacturer's directions. 10-20 $\mu$ g RNA from each clone was analyzed on duplicate formaldehyde gels. The resulting blots were probed with PCR  
10       generated digoxigenin labelled DNA probes to (i) DHFR message, (ii) HisD message and (iii) CAD message. CAD is a trifunctional protein involved in uridine biosynthesis (Wahl et al, *J. Biol. Chem.*, 254, 17:8679-8689 (1979)), and is expressed equally in all cell  
15       types. It is used here as an internal control to help quantitate RNA loading. Hybridizations and washes were carried out using the above mentioned Boehringer Mannheim reagents. The results of the Northern analysis are shown in Figure 5. The single copy Desmond clone  
20       exhibiting the highest levels of both the His D and DHFR message is clone 15C9, shown in lane 4 in both panels of the figure. This clone was designated as the "marked cell line" and used in future targeting experiments in CHO, examples of which are presented in the following  
25       sections.

- 34 -

EXAMPLE 4Expression of Anti-CD20 Antibody  
in Desmond Marked CHO Cells

C2B8, a chimeric antibody which recognizes B-cell  
5 surface antigen CD20, has been cloned and expressed  
previously in our laboratory. (Reff et al, *Blood*,  
83:434-45 (1994)). A 4.1 kb DNA fragment comprising the  
C2B8 light and heavy chain genes, along with the neces-  
sary regulatory elements (eukaryotic promoter and poly-  
10 adenylation signals) was inserted into the artificial  
intron created between exons 1 and 2 of the neo gene  
contained in a pBR derived cloning vector. This newly  
generated 5kb DNA fragment (comprising neo exon 1, C2B8  
and neo exon 2) was excised and used to assemble the  
15 targeting plasmid Molly. The other DNA elements used in  
the construction of Molly are identical to those used to  
construct the marking plasmid Desmond, identified  
previously. A complete map of Molly is shown in Fig. 2.

The targeting vector Molly was linearized prior to  
20 transfection by digestion with *Kpn*I and *Pac*I, ethanol  
precipitated and resuspended in sterile TE to a concen-  
tration of 1.5mg/mL. Linearized plasmid was introduced  
into exponentially growing Desmond marked cells essen-  
tially as described, except that 80µg DNA was used in  
25 each electroporation. Forty eight hours postelectropo-  
ration, 96 well plates were supplemented with selection  
medium - CHO-SSFMI supplemented with 400 µg/mL Geneti-

- 35 -

cin (G418, Gibco/BRL catalog # 10131-019). Plates were maintained in selection medium for up to 30 days, or until cell growth occurred in some of the wells. Such growth was assumed to be the result of clonal expansion of a single G418 resistant cell. The supernatants from all G418 resistant wells were assayed for C2B8 production by standard ELISA techniques, and all productive clones were eventually expanded to 120mL spinner flasks and further analyzed.

10 Characterization of Antibody secreting Targeted Cells

A total of 50 electroporations with Molly targeting plasmid were carried out in this experiment, each of which was plated into separate 96 well plates. A total of 10 viable, anti-CD20 antibody secreting clones were obtained and expanded to 120ml spinner flasks. Genomic DNA was isolated from all clones, and Southern analyses were subsequently performed to determine whether the clones represented single homologous recombination events or whether additional random integrations had occurred in the same cells. The methods for DNA isolation and Southern hybridization were as described in the previous section. Genomic DNA was digested with EcoRI and probed with a PCR generated digoxigenin labelled probe to a segment of the CD20 heavy chain constant region. The results of this Southern analysis are presented in figure 6. As can be seen in the figure, 8 of

- 36 -

the 10 clones show a single band hybridizing to the CD20 probe, indicating a single homologous recombination event has occurred in these cells. Two of the ten, clones 24G2 and 28C9, show the presence of additional band(s), indicative of an additional random integration elsewhere in the genome.

We examined the expression levels of anti-CD20 antibody in all ten of these clones, the data for which is shown in Table 1, below.

Table 1:

Expression Level of Anti-CD20  
Secreting Homologous Integrants

<u>Clone</u>	<u>Anti-CD20, pg/c/d</u>
20F4	3.5
25E1	2.4
42F9	1.8
39G11	1.5
21C7	1.3
50G10	0.9
29F9	0.8
5F9	0.3
-----	
28C9*	4.5
24G2*	2.1

- 37 -

\* These clones contained additional randomly integrated copies of anti-CD20. Expression levels of these clones therefore reflect a contribution from both the homologous and random sites.

5

Expression levels are reported as picogram per cell per day (pg/c/d) secreted by the individual clones, and represented the mean levels obtained from three separate ELISAs on samples taken from 120 mL spinner flasks.

10

As can be seen from the data, there is a variation in antibody secretion of approximately ten fold between the highest and lowest clones. This was somewhat unexpected as we anticipated similar expression levels from all clones due to the fact the anti-CD20 genes are all integrated into the same Desmond marked site. Nevertheless, this observed range in expression extremely small in comparison to that seen using any traditional random integration method or with our translationally impaired vector system.

15

20

Clone 20F4, the highest producing single copy integrant was selected for further study. Table 2 (below) presents ELISA and cell culture data from seven day production runs of this clone.

- 38 -

Table 2:

## 7 Day Production Run Data for 20F4

Day	% Viable	Viable/ml (x 10 <sup>5</sup> )	Tx2(hr)	mg/L	pg/c/d
1	96	3.4	31	1.3	4.9
2	94	6	29	2.5	3.4
3	94	9.9	33	4.7	3.2
4	90	17.4	30	6.8	3
5	73	14		8.3	
6	17	3.5		9.5	

Clone 20F4 was seeded at  $2 \times 10^5$  ml in a 120ml spinner flask on day 0. On the following six days, cell counts were taken, doubling times calculated and 1ml samples of supernatant removed from the flask and analyzed for secreted anti-CD20 by ELISA.

This clone is secreting on average, 3-5pg antibody/-cell/day, based on this ELISA data. This is the same level as obtained from other high expressing single copy clones obtained previously in our laboratory using the previously developed translationally impaired random integration vectors. This result indicates the following:

(1) that the site in the CHO genome marked by the Desmond marking vector is highly transcriptionally active, and therefore represents an excellent site from which to express recombinant proteins, and

- 39 -

(2) that targeting by means of homologous recombination can be accomplished using the subject vectors and occurs at a frequency high enough to make this system a viable and desirable alternative to random integration methods.

To further demonstrate the efficacy of this system, we have also demonstrated that this site is amplifiable, resulting in even higher levels of gene expression and protein secretion. Amplification was achieved by plating serial dilutions of 20F4 cells, starting at a density of  $2.5 \times 10^4$  cells/ml, in 96 well tissue culture dishes, and culturing these cells in media (CHO-SSFMII) supplemented with 5, 10, 15 or 20nM methotrexate. Antibody secreting clones were screened using standard ELISA techniques, and the highest producing clones were expanded and further analyzed. A summary of this amplification experiment is presented in Table 3 below.

- 40 -

Table 3:

## Summary of 20F4 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
10	56	3-13	4	10-15
15	27	2-14	3	15-18
20	17	4-11	1	ND

Methotrexate amplification of 20F4 was set up as described in the text, using the concentrations of methotrexate indicated in the above table. Supernatants from all surviving 96 well colonies were assayed by ELISA, and the range of anti-CD20 expressed by these clones is indicated in column 3. Based on these results, the highest producing clones were expanded to 120ml spinners and several ELISAs conducted on the spinner supernatants to determine the pg/cell/day expression levels, reported in column 5.

The data here clearly demonstrates that this site can be amplified in the presence of methotrexate. Clones from the 10 and 15nM amplifications were found to produce on the order of 15-20pg/cell/day.

A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell.

A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated

- 41 -

from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell. The clone was then subjected to a further round of methotrexate amplification. As described above, serial dilutions of the culture were plated into 96 well dishes and cultured in CHO-SS-FMII medium supplemented with 200, 300 or 400nM methotrexate. Surviving clones were screened by ELISA, and several high producing clones were expanded to spinner cultures and further analyzed. A summary of this second amplification experiment is presented in Table 4.

Table 4:

## Summary of 20F4-15A5 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d, spinner
200	67	23-70	1	50-60
250	86	21-70	4	55-60
300	81	15-75	3	40-50

Methotrexate amplifications of 20F4-15A5 were set up and assayed as described in the text. The highest producing wells, the numbers of which are indicated in column 4, were expanded to 120ml spinner flasks. The expression levels of the cell lines derived from these wells is recorded as pg/c/d in column 5.

The highest producing clone came from the 250nM methotrexate amplification. The 250nM clone, 20F4-15A5-250A6 originated from a 96 well plate in which only wells

- 42 -

grew, and therefore is assumed to have arisen from a single cell. Taken together, the data in Tables 3 and 4 strongly indicates that two rounds of methotrexate amplification are sufficient to reach expression levels of 5 60pg/cell/day, which is approaching the maximum secretion capacity of immunoglobulin in mammalian cells (Reff, M.E., *Curr. Opin. Biotech.*, 4:573-576 (1993)). The ability to reach this secretion capacity with just two amplification steps further enhances the utility of 10 this homologous recombination system. Typically, random integration methods require more than two amplification steps to reach this expression level and are generally less reliable in terms of the ease of amplification. Thus, the homologous system offers a more efficient and 15 time saving method of achieving high level gene expression in mammalian cells.

#### EXAMPLE 5

##### Expression of Anti-Human CD23 Antibody in Desmond Marked CHO Cells

20 CD23 is low affinity IgE receptor which mediates binding of IgE to B and T lymphocytes (Sutton, B.J., and Gould, H.J., *Nature*, 366:421-428 (1993)). Anti-human CD23 monoclonal antibody 5E8 is a human gamma-1 monoclonal antibody recently cloned and expressed in our 25 laboratory. This antibody is disclosed in commonly

- 43 -

assigned Serial No. 08/803,085, filed on February 20, 1997.

5 The heavy and light chain genes of 5E8 were cloned into the mammalian expression vector N5KG1, a derivative of the vector NEOSPLA (Barnett et al, in *Antibody Expression and Engineering*, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)) and two modifications were then made to the genes. We have recently observed somewhat higher secretion of immunoglobulin light chains compared to heavy chains in other expression constructs in the laboratory (Reff et al, 1997, unpublished observations). In an attempt to compensate for this deficit, we altered the 5E8 heavy chain gene by the addition of a stronger promoter/enhancer element immediately upstream of the start site. In subsequent steps, a 2.9kb DNA fragment comprising the 5E8 modified light and heavy chain genes was isolated from the N5KG1 vector and inserted into the targeting vector Mandy. Preparation of 5E8-containing Molly and electroporation into Desmond 15C9 CHO cells was essentially as described in the preceding section.

20 One modification to the previously described protocol was in the type of culture medium used. Desmond marked CHO cells were cultured in protein-free CD-CHO medium (Gibco-BRL, catalog # AS21206) supplemented with 3mg/L recombinant insulin (3mg/mL stock, Gibco-BRL, catalog # AS22057) and 8mM L-glutamine (200mM stock, Gibco-BRL, catalog # 25030-081). Subsequently, trans-

- 44 -

fectected cells were selected in the above medium supplemented with 400 $\mu$ g/mL geneticin. In this experiment, 20 electroporations were performed and plated into 96 well tissue culture dishes. Cells grew and secreted anti-  
5 CD23 in a total of 68 wells, all of which were assumed to be clones originating from a single G418 cell. Twelve of these wells were expanded to 120ml spinner flasks for further analysis. We believe the increased number of clones isolated in this experiment (68 compared with 10 for anti-CD20 as described in Example 4) is due to a higher cloning efficiency and survival rate of cells grown in CD-CHO medium compared with CHO-SS-FMII medium. Expression levels for those clones analyzed in spinner culture ranged from 0.5-3pg/c/d, in  
10 close agreement with the levels seen for the anti-CD20 clones. The highest producing anti-CD23 clone, designated 4H12, was subjected to methotrexate amplification in order to increase its expression levels. This amplification was set up in a manner similar to that described for the anti-CD20 clone in Example 4. Serial dilutions of exponentially growing 4H12 cells were plated into 96 well tissue culture dishes and grown in CD-CHO medium supplemented with 3mg/L insulin, 8mM glutamine and 30, 35 or 40nM methotrexate. A summary of this  
15 amplification experiment is presented in Table 5.

Table 5:

- 45 -

## Summary of 2H12 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
30	100	6-24	8	10-25
35	64	4-27	2	10-15
40	96	4-20	1	ND

The highest expressing clone obtained was a 30nM clone, isolated from a plate on which 22 wells had grown. This clone, designated 4H12-30G5, was reproducibly secreting 18-22pg antibody per cell per day. This is the same range of expression seen for the first amplification of the anti CD20 clone 20F4 (clone 20F4-15A5 which produced 15-18pg/c/d, as described in Example 4). This data serves to further support the observation that amplification at this marked site in CHO is reproducible and efficient. A second amplification of this 30nM cell line is currently underway. It is anticipated that saturation levels of expression will be achievable for the anti-CD23 antibody in just two amplification steps, as was the case for anti-CD20.

EXAMPLE 6Expression of Immunoadhesin in Desmond Marked CHO Cells

CTLA-4, a member of the Ig superfamily, is found on the surface of T lymphocytes and is thought to play a role in antigen-specific T-cell activation (Dariavach et al, *Eur. J. Immunol.*, 18:1901-1905 (1988); and Linsley et al, *J. Exp. Med.*, 174:561-569 (1991)). In order to further study the precise role of the CTLA-4 molecule in the activation pathway, a soluble fusion protein comprising the extracellular domain of CTLA-4 linked to a truncated form of the human IgG1 constant region was

- 46 -

created (Linsley et al (Id.)). We have recently expressed this CTLA-4 Ig fusion protein in the mammalian expression vector BLECH1, a derivative of the plasmid NEOSPLA (Barnett et al, in Antibody Expression and Engineering, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)).  
5 An 800bp fragment encoding the CTLA-4 Ig was isolated from this vector and inserted between the SacII and BglIII sites in Molly.

Preparation of CTLA-4Ig-Molly and electroporation  
10 into Desmond clone 15C9 CHO cells was performed as described in the previous example relating to anti-CD20. Twenty electroporations were carried out, and plated into 96 well culture dishes as described previously. Eighteen CTLA-4 expressing wells were isolated from the  
15 96 well plates and carried forward to the 120ml spinner stage. Southern analyses on genomic DNA isolated from each of these clones were then carried out to determine how many of the homologous clones contained additional random integrants. Genomic DNA was digested with BglIII  
20 and probed with a PCR generated digoxigenin labelled probe to the human IgG1 constant region. The results of this analysis indicated that 85% of the CTLA-4 clones are homologous integrants only; the remaining 15% contained one additional random integrant. This result  
25 corroborates the findings from the expression of anti-CD20 discussed above, where 80% of the clones were single homologous integrants. Therefore, we can conclude

- 47 -

that this expression system reproducibly yields single targeted homologous integrants in at least 80% of all clones produced.

Expression levels for the homologous CT1A4-Ig clones ranged from 8-12pg/cell/day. This is somewhat higher than the range reported for anti-CD20 antibody and anti-CD23 antibody clones discussed above. However, we have previously observed that expression of this molecule using the intronic insertion vector system also resulted in significantly higher expression levels than are obtained for immunoglobulins. We are currently unable to provide an explanation for this observation.

#### EXAMPLE 7

##### Targeting Anti-CD20 to an alternate Desmond Marked CHO Cell Line

As we described in a preceding section, we obtained 5 single copy Desmond marked CHO cell lines (see Figures 4 and 5). In order to demonstrate that the success of our targeting strategy is not due to some unique property of Desmond clone 15C9 and limited only to this clone, we introduced anti-CD20 Molly into Desmond clone 9B2 (lane 6 in figure 4, lane 1 in figure 5). Preparation of Molly DNA and electroporation into Desmond 9B2 was exactly as described in the previous example pertaining to anti-CD20. We obtained one homologous integrant from this experiment. This clone was expanded to a 120ml

- 48 -

spinner flask, where it produced on average 1.2pg anti-CD20/cell/day. This is considerably lower expression than we observed with Molly targeted into Desmond 15C9. However, this was the anticipated result, based on our  
5 northern analysis of the Desmond clones. As can be seen in Figure 5, mRNA levels from clone 9B2 are considerably lower than those from 15C9, indicating the site in this clone is not as transcriptionally active as that in 15C9. Therefore, this experiment not only demonstrates  
10 the reproducibility of the system - presumably any marked Desmond site can be targeted with Molly - it also confirms the northern data that the site in Desmond 15C9 is the most transcriptionally active.

From the foregoing, it will be appreciated that,  
15 although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without diverting from the scope of the invention. Accordingly, the invention is not limited by the appended claims.

- 49 -

WHAT IS CLAIMED IS:

1. A method for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises the following steps:

5 (i) transfecting or transforming a mammalian cell with a first plasmid ("marker plasmid") containing the following sequences:

(a) a region of DNA that is heterologous to the mammalian cell genome which when integrated in the  
10 mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first selectable marker protein; and

(c) at least one other selectable marker DNA  
15 that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid;

(ii) selecting a cell which contain the marker plasmid integrated in its genome;

20 (iii) transfecting or transforming said selected cell with a second plasmid ("target plasmid") which contains the following sequences:

(a) a region of DNA that is identical or is sufficiently homologous to the unique region in the  
25 marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

- 50 -

(b) a DNA fragment encoding a portion of the same selectable marker contained in the marker plasmid, wherein the active selectable marker protein encoded by said DNA is only produced if said fragment is expressed  
5 in association with the fragment of said selectable marker DNA contained in the marker plasmid; and

(iv) selecting cells which contain the target plasmid integrated at the target site by screening for the expression of the first selectable marker protein.

10 2. The method of Claim 1, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

3. The method of Claim 2, wherein the second plasmid contains the remaining exons of said first  
15 selectable marker.

4. The method of Claim 3, wherein at least one DNA encoding a desired protein is inserted between said exons of said first selectable marker contained in the target plasmid.

20 5. The method Claim 4, wherein a DNA encoding a dominant selectable marker is further inserted between the exons of said first selectable marker contained in

- 51 -

the target plasmid to provide for co-amplification of the DNA encoding the desired protein.

6. The method of Claim 3, wherein the first dominant selectable marker is selected from the group consisting of neomycin phosphotransferase, histidinol dehydrogenase, dihydrofolate reductase, hygromycin phosphotransferase, herpes simplex virus thymidine kinase, adenosine deaminase, glutamine synthetase, and hypoxanthine-guanine phosphoribosyl transferase.

7. The method of Claim 4, wherein the desired protein is a mammalian protein.

8. The method of Claim 7, wherein the protein is an immunoglobulin.

9. The method of Claim 1, which further comprises determining the RNA levels of the selectable marker (c) contained in the marker plasmid prior to integration of the target vector.

10. The method of Claim 9, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex

- 52 -

thymidine kinase, hydromycin phosphotransferase, adenosine deaminase and glutamine synthetase.

11. The method of Claim 1, wherein the mammalian cell is selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

12. The method of Claim 11, wherein the cell is a CHO cell.

13. The method of Claim 1, wherein the marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains the first two exons of the neomycin phosphotransferase gene.

14. The method of Claim 1, wherein the marker plasmid further contains a rare restriction endonuclease sequence which is inserted within the region of homology.

15. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic DNA.

- 53 -

16. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is at least 300 nucleotides.

17. The method of Claim 16, wherein the unique region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

18. The method of claim 17, wherein the unique region of DNA preferably ranges in size from 2 to 10 kilobases.

19. The method of Claim 1, wherein the first selectable marker DNA is split into at least three exons.

20. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

21. The method of Claim 20, wherein the unique region of DNA does not contain any functional genes.

22. A vector system for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises at least the following:

- 54 -

(i) a first plasmid ("marker plasmid") containing at least the following sequences:

(a) a region of DNA that is heterologous to the mammalian cell genome which when integrated in the mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first selectable marker protein; and

(c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid; and

(ii) a second plasmid ("target plasmid") which contains at least the following sequences:

(a) a region of DNA that is identical or is sufficiently homologous to the unique region in the marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

(b) a DNA fragment encoding a portion of the same selectable marker contained in the marker plasmid, wherein the active selectable marker protein encoded by said DNA is only produced if said fragment is expressed in association with the fragment of said selectable marker DNA contained in the marker plasmid.

- 55 -

23. The vector system of Claim 22, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

24. The vector system of Claim 23, wherein the  
5 second plasmid contains the remaining exons of said first selectable marker.

25. The vector system of Claim 24, wherein at least one DNA encoding a desired protein is inserted between said exons of said first selectable marker con-  
10 tained in the target plasmid.

26. The vector system of Claim 24, wherein a DNA encoding a dominant selectable marker is further inserted between the exons of said first selectable marker contained in the target plasmid to provide for co-ampli-  
15 fication of the DNA encoding the desired protein.

27. The vector system of Claim 24, wherein the first dominant selectable marker is selected from the group consisting of neomycin phosphotransferase, histidinol dehydrogenase, dihydrofolate reductase,  
20 hygromycin phosphotransferase, herpes simplex virus thymidine kinase, adenosine deaminase, glutamine synthetase, and hypoxanthine-guanine phosphoribosyl transferase.

- 56 -

28. The vector system of Claim 25, wherein the desired protein is a mammalian protein.

29. The vector system of Claim 28, wherein the protein is an immunoglobulin.

5       30. The vector system of Claim 22, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex thymidine kinase, hydromycin phosphotransferase, adeno-  
10   sine deaminase and glutamine synthetase.

31. The vector system of Claim 22, which provides for insertion of a desired DNA at a targeted site in the genome of a mammalian cell selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma  
15   cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

32. The vector system of Claim 31, wherein the mammalian cell is a CHO cell.

33. The vector system of Claim 22, wherein the  
20   marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains

- 57 -

the first two exons of the neomycin phosphotransferase gene.

34. The vector system of Claim 22, wherein the marker plasmid further contains a rare restriction endo-  
5 nuclease sequence which is inserted within the region of homology.

35. The vector system of Claim 22, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic  
10 DNA.

36. The vector system of Claim 22, wherein the unique region of DNA (a) contained in the marker plasmid vector system that provides for homologous recombination is at least 300 nucleotides.

15 37. The vector system of Claim 36, wherein the unique region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

38. The vector system of Claim 37, wherein the unique region of DNA preferably ranges in size from 2 to  
20 10 kilobases.

- 58 -

39. The vector system of Claim 22, wherein the first selectable marker DNA is split into at least three exons.

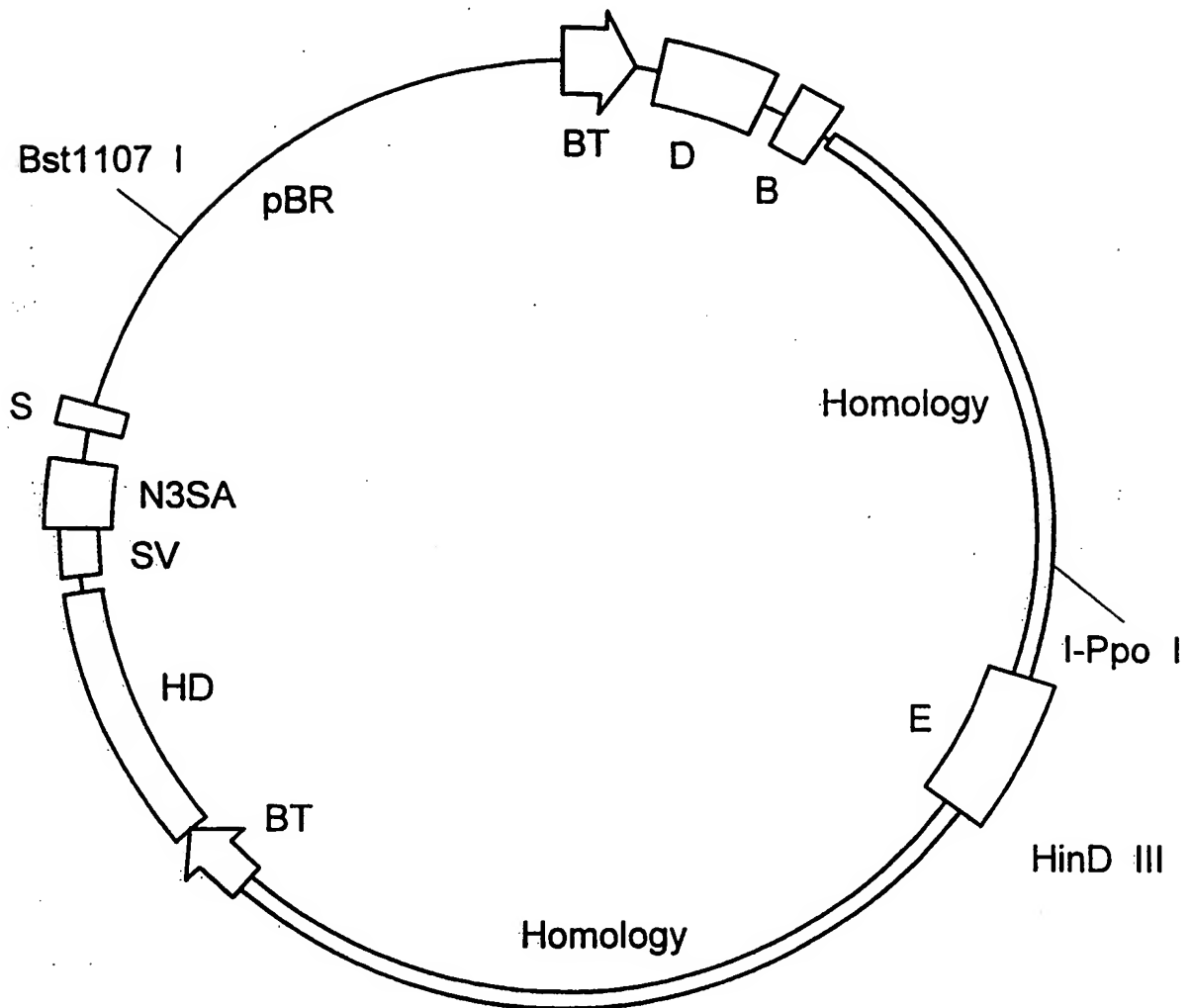
40. The vector system of Claim 22, wherein the  
5 unique region of DNA that provides for homologous recombination is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

41. The vector system of Claim 40, wherein the  
10 unique region of DNA does not contain any functional genes.

1/75

# FIG. 1A

## DESMOND



HD = Salmonella HisD Gene  
 N3 = Neomycin Phosphotransferase Exon 3  
 D = Murine Dihydrofolate reductase  
 E = Cytomegalovirus and SV40 Enhancers  
 SA = Splice acceptor  
 BT = Mouse Beta Globin Major Promoter  
 B = Bovine Growth Hormone Polyadenylation  
 S = SV40 Early Polyadenylation  
 SV = SV40 Late Polyadenylation

Desmond  
14,683 bp Bst1107 I linear

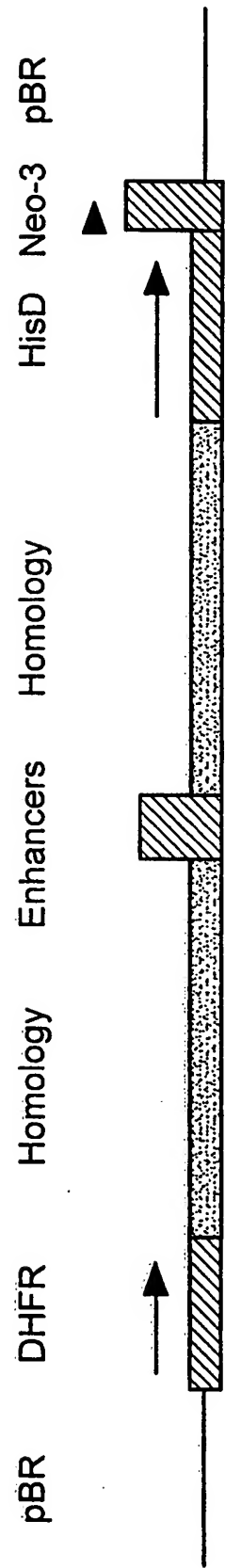
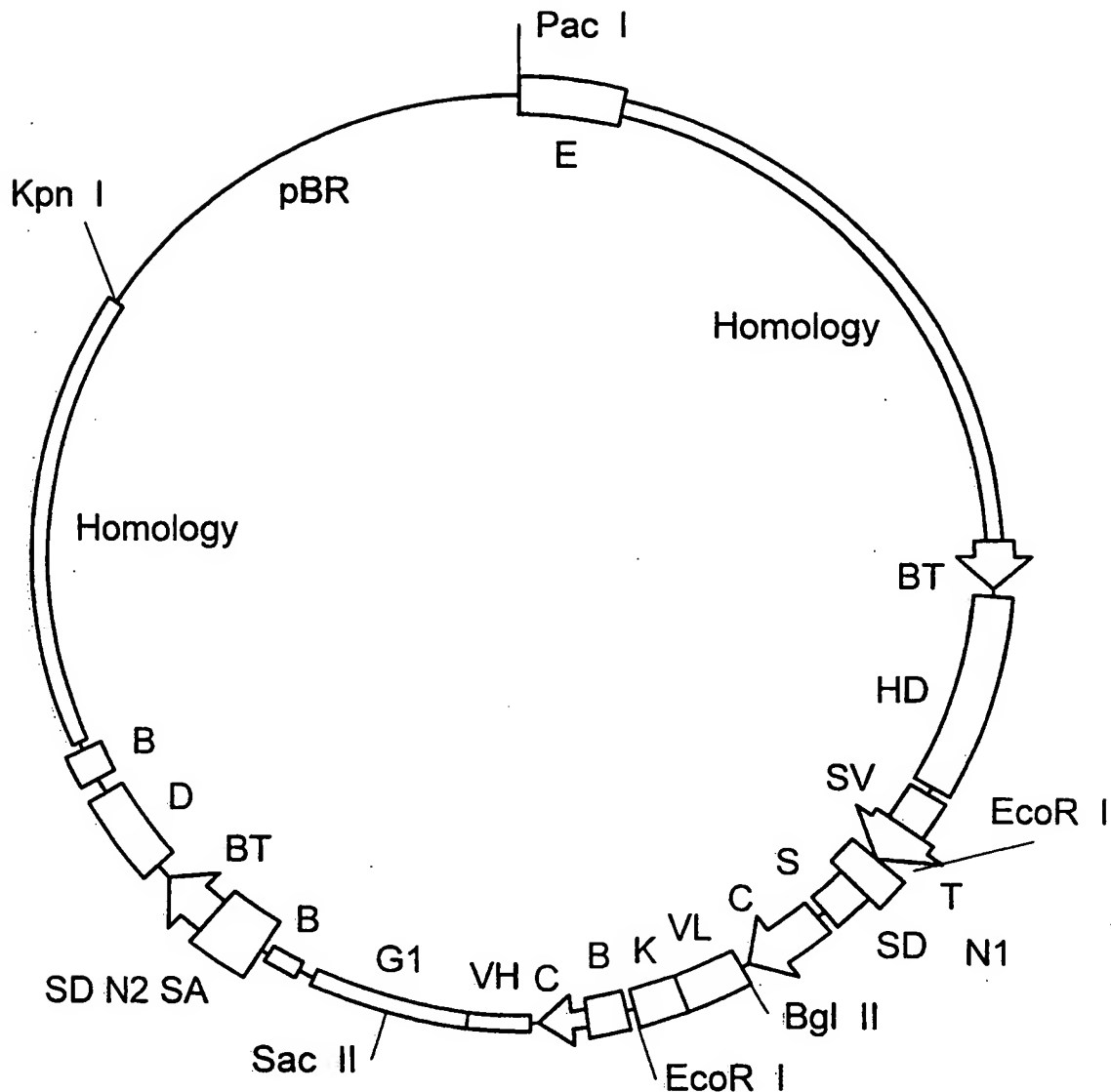


FIG. 1B

3/75

Molly

FIG. 2A



- D = Dihydrofolate reductase
- N1 + Neomycin Phosphotransferase Exon 1
- N2 + Neomycin Phosphotransferase Exon 2
- VL = Anti-CD20 Light chain leader + Variable
- K = Human Kappa Constant
- VH = Anti-CD20 Heavy chain Leader + Variable
- G1 = Human Gamma 1 Constant
- HD = Salmonella Histidinol Dehydrogenase
- E = CMV and SV40 enhancers      S = SV40 Origin
- SD = Splice donor      SA = Splice acceptor
- C = CMV promoter/enhancer
- T = HSV TK promoter and Poloma enhancers
- BT = Mouse Beta Globin Major Promoter
- SV = SV40 Late Polyadenylation
- B = Bovine Growth Hormone Polyadenylation

4/75

Molly  
15,987 bp Pac I, Kpn I fragment

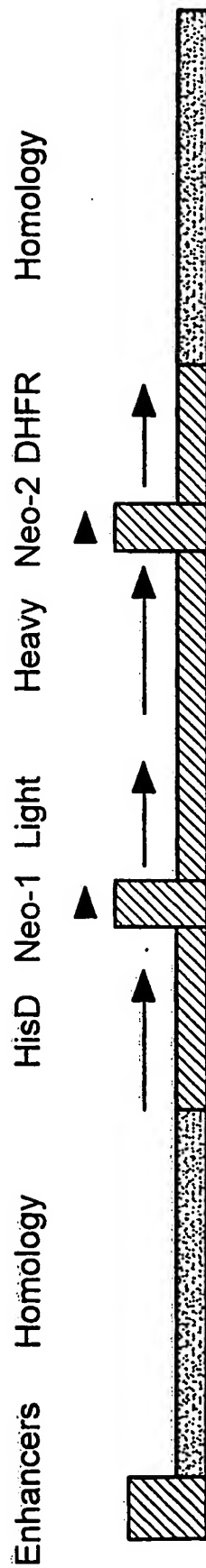
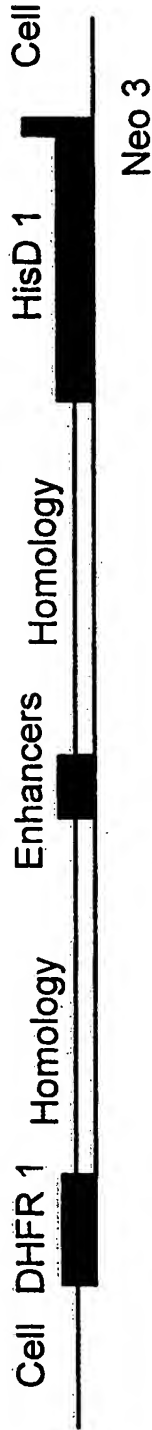


FIG. 2B

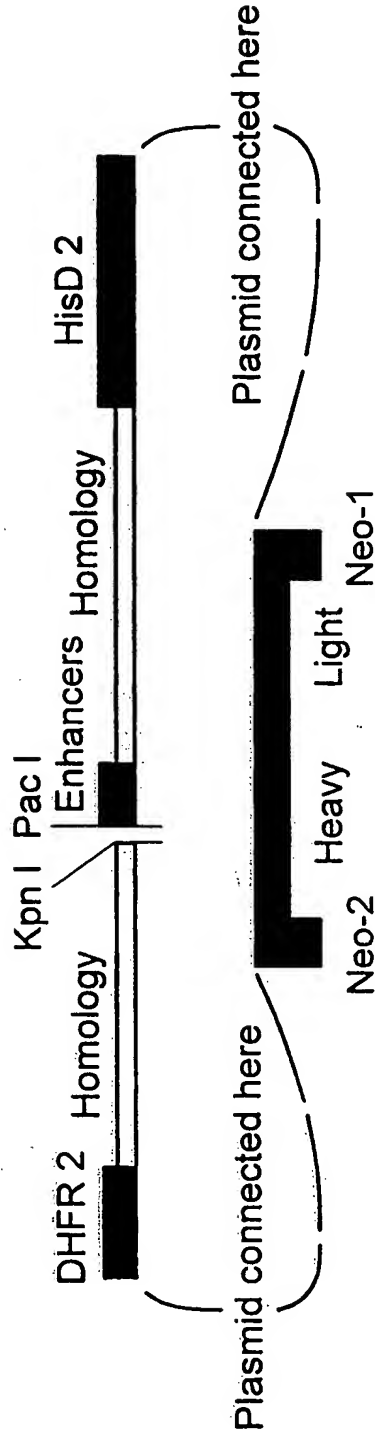
FIG. 3

# Homologous Recombination

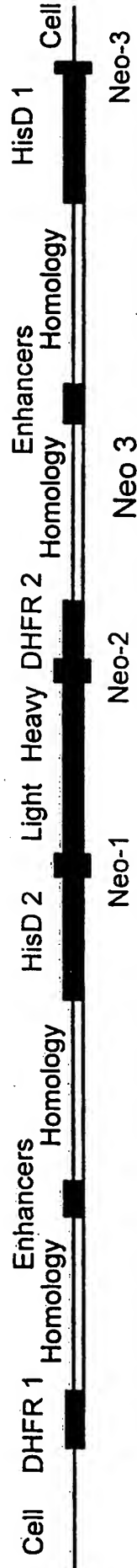
## Desmond in CHO



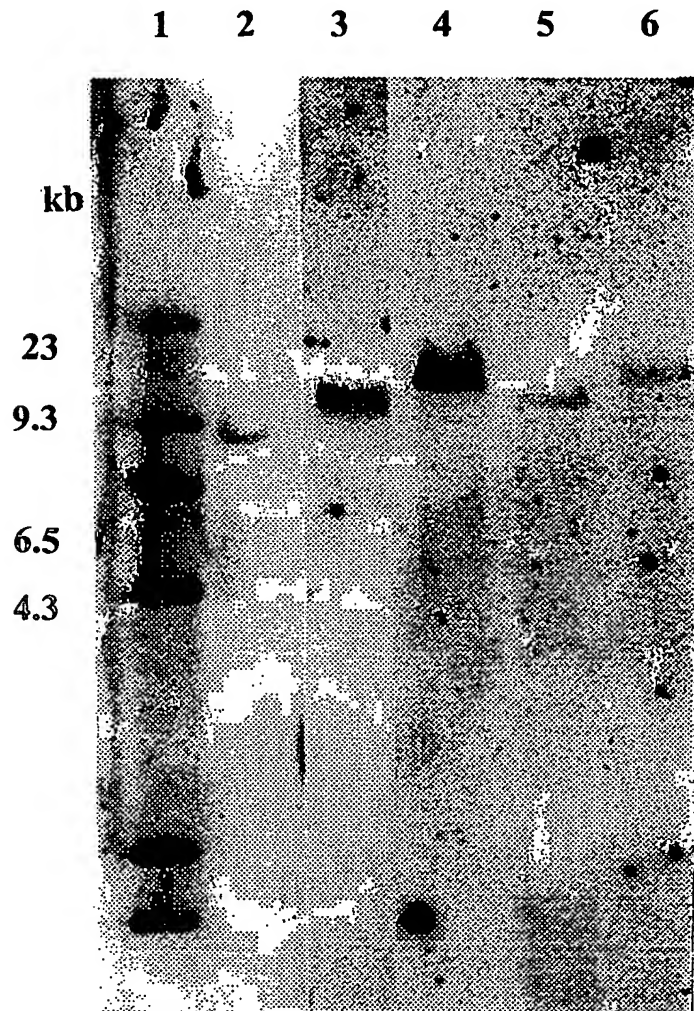
## Molly



## Single crossover in CHO

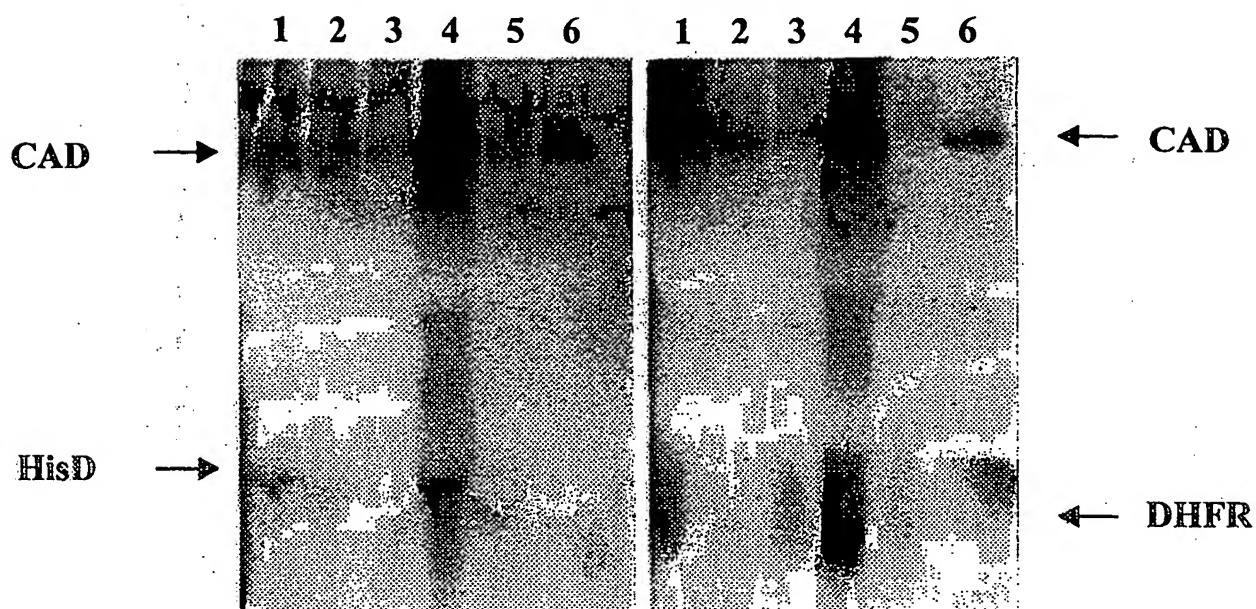


6/75

**Southern Analysis of Desmond Marked CHO Cells****FIG. 4**

7/75

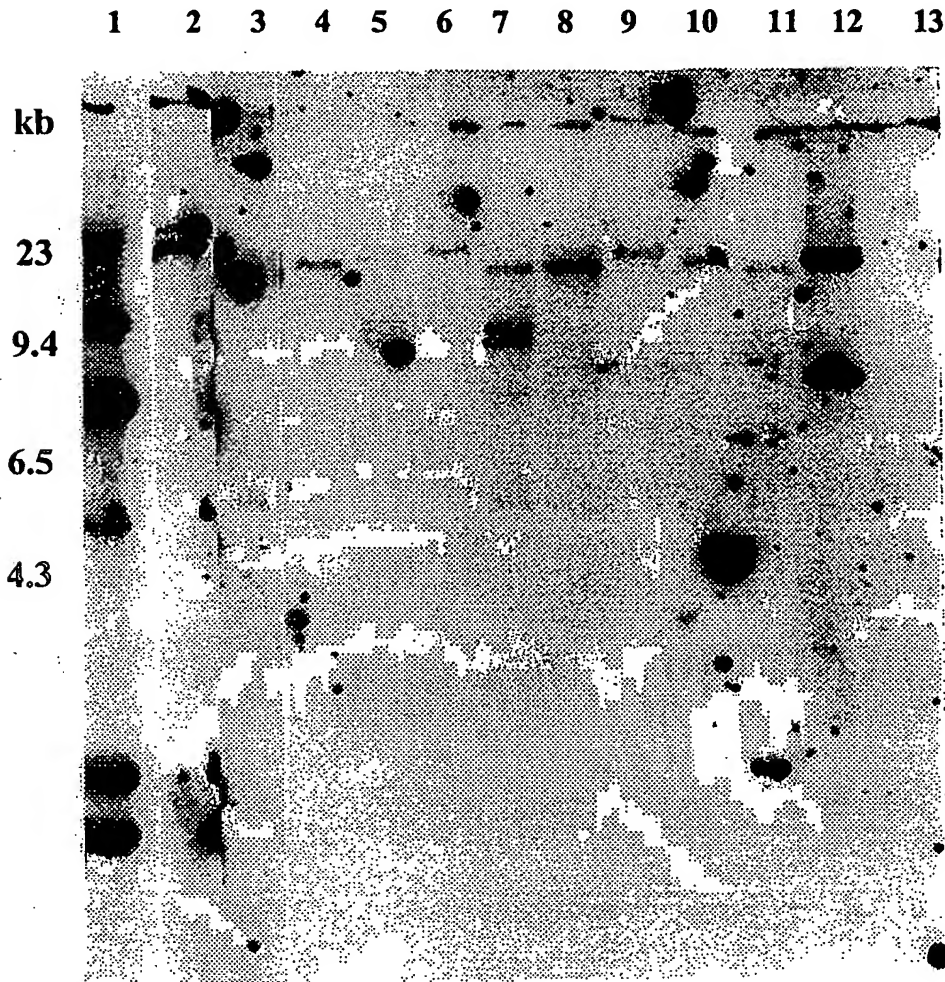
**Northern Analysis of Desmond**  
**Marked CHO Cells**



**FIG. 5**

8/75

**Southern Analysis of Anti CD20**  
**Integrants in Marked CHO Cells**



**FIG. 6**

## WV 20141043

FC 1/US98/US935

# FIG. 7B

AAACCTGGTT CTCCATTCCCT GAGAAGAATC GACCTTTTAA GGACAGAATT AATATAGTTC 720  
TCAGTAGAGA ACTCAAAGAA CCACCACGAG GAGCTCATTT TCTTGCCCAA AGTTTGGATG 780  
ATGCCTTAAG ACTTATTGAA CAACCGGAAT TGGCAAGTAA AGTAGACATG GTTTGGATAG 840  
TCGGAGGCAG TTCTGTTTAC CAGGAAGCCA TGAATCAACC AGGCCACCCT AGACTCTTTG 900  
TGACAAGGAT CATGCAGGAA TTTGAAAGTG ACACGTTTTT CCCAGAAATT GATTTGGGGA 960  
AATATAAACT TCTCCCAGAA TACCCAGGCG TCCTCTCTGA GTCCAGGAG GAAAAGGCA 1020  
TCAAGTATAA GTTTGAAGTC TACGAGAAGA AAGACTAACA GGAAGATGCT TTCAAGTTCT 1080  
CTGCTCCCCCT CCTAAAGCTA TGCATTTTAA TAAGACCATG GGACTTTTGC TGGCTTTAGA 1140  
TCAGCCTCGA CTGTGCCTTC TAGTTGCCAG CCATCTGTTG TTTGCCCCCTC CCCC GTGCCT 1200  
TCCTTGACCC TGGAAGGTGC CACTCCCCT GTCCCTTTCCT AATAAAATGA GGAAATTGCA 1260  
TCGCATTGTC TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG 1320

# FIG. 7C

GGGAGGATT GGAAGACAA TAGCAGGCAT GCTGGGGATG CCGTGGGCTC TATGGAACCA 1380  
GCTGGGGCTC GAAGCGGCCG CCCATTTCCG TGGTGGTCAG ATCGGGGATG GCGTGGGACG 1440  
CGCGGGGAC CGTCACACTG AGGTTTTCCG CCAGACGCCA CTGCTGCCAG GCGCTGATGT 1500  
GCCCCGGCTT TGACCATGCG GTCGCGTTCC GTTGCACTAC GCGTACTGTG AGCCAGAGTT 1560  
GCCCCGGGCT CTCCGGGCTG GGTAGTTCAG GCAGTTCAAT CAACTGTTTA CCTTGTGGAG 1620  
CGACATCCAG AGGCATTCA CCGCTTGCTA GCGGCTTACC ATCCAGCGCC ACCATCCAGT 1680  
GCAGGAGCTC GTTATCGCTA TGACGGAACA GGTATTGCT GGTCACTTCG ATGGTTTGCC 1740  
CGGATAAACG GAACTGGAAA AACTGCTGCT GGTGTTTTGC TTCCGTCAGC GCTGGATGCG 1800  
GCGTGCGGTC GGCAAAGACC AGACCGTTCA TACAGAACTG GCGATCGTTC GCGGTATCAC 1860  
CAAAATCACC GCCGTAAGCC GACCACGGGT TGCCGTTTTTTC ATCATATTTA ATCAGCGACT 1920  
GATCCACCCA GTCCCAGACG AAGCCGCCCT GTAAACGGGG ATACTGACGA AACGCCCTGCC 1980

# FIG. 7D

AGTATTTAGC GAAACCGCCA AGACTGTTAC CCATCGCGTG GCGGTATTTC CAAAGGATCA 2040  
GCGGGCGCGT CTCTCCGGGT AGCGAAAGCC ATTTTGTGAT GGACCATTTC GGACCAGCCG 2100  
GGAAGGGCTG GTCCTTCATCC ACGCGCGCGT ACATCGGGCA AATAATATCG GTGGCCGTGG 2160  
TGTCGGCTCC GCCGCCCTTCA TACTGCACCG GCGGGGAAGG ATCGACAGAT TTGATCCAGC 2220  
GATACAGCGC GTCGTGATTA GCGCCGTGGC CTGATTTCATT CCCACGCGAC CAGATGATCA 2280  
CACTCGGGTG ATTACGATCG CGCTGCACCA TTCGCGTTAC GCGTTCGCTC ATCGCCGGTA 2340  
GCCAGCGCGG ATCATCGGTC AGACGATTCA TTGGCACCAT GCCGTGGGT TCAATATTGG 2400  
CTTCATCCAC CACATACAGG CCGTAGCGGT CGCACAGCGT GTACCACAGC GGATGGTTTCG 2460  
GATAATGCGA ACAGCGCAGC GCGTTAAAGT TGTTCTGCTT CATCAGCAGG ATATCCTGCA 2520  
CCATCGTCTG CTCATCCATG ACCTGACCAT GCAGAGGATG ATGCTCGTGA CGGTTAACGC 2580  
CTCGAATCAG CAACGGCTTG CCGTTCAGCA GCAGCAGACC ATTCCAATC CGCACCTCGC 2640

13/75

GGAAACCGAC ATCGCAGGCT TCTGCTTCAA TCAGCGTGCC GTCGGCGGTG TGCAGTTCAA 2700

CCACCGCACG ATAGAGATTC GGGATTTCGG CGCTCCACAG TTTCGGGTTT TCGACGTTCA 2760

GACGCAGTGT GACGCGATCG GCATAACCAC CAGGCTCATC GATAATTTCA CCGCCGAAAG 2820

GCGCGGTGCC GCTGGCGACC TCGGTTTCAC CCTGCCATAA AGAAACTGTT ACCCGTAGGT 2880

AGTCACGCAA CTCGCCGCAC ATCTGAACTT CAGCCTCCAG TACAGCGCGG CTGAAATCAT 2940

CATTAAAGCG AGTGGAACA TGGAAATCGC TGATTTGTGT AGTCGGTTTA TGCAGCAACG 3000

AGACGTCACG GAAATGCCG CTCATCCGCC ACATATCCTG ATCTTCCAGA TAACTGCCCGT 3060

CACTCCAACG CAGCACCATC ACCGCGAGGC GGTTTCTCC GGCGCGTAA AATGCGCTCA 3120

GGTCAAATTC AGACGGCAA CGACTGTCCT GGCTGTAACC GACCCACGCC CCGTTGCACC 3180

ACAGATGAAA CGCCGAGTTA ACGCCATCAA AAATAATTCC CGTCTGGCCT TCCTGTAGCC 3240

AGCTTTCATC AACATTAAAT GTGAGCGAGT ACAAACCCGT CGGATTCTCC GTGGGAACAA 3300

# FIG. 7F

ACGGCGGATT GACCGTAATG GGATAGGTTA CGTTGGTGTA GATGGGGCGCA TCGTAACCGT 3360  
GCATCTGCCA GTTTGAGGGG ACGACGACAG TATCGGCCTC AGGAAGATCG CACTCCAGCC 3420  
AGCTTTCCGG CACTGCTTCT GGTGCCGGAA ACCAGGCATA GCGCCATTCC CCATTACAGG 3480  
TGCGCAACTG TTGGGAAGGG CGATCGGTGC GGGCCTCTTC GCTATTACGC CAGCTGGCGA 3540  
AAGCGGGATG TGCTGCAAGG CGATTAAATT GGGTAACGCC AGGGTTTTCC CAGTCACGAC 3600  
GTTGTAAAC GACTTAATCC GTCGAGGGG TGCCTCGAAG CAGACGACCT TCCGTTGTGC 3660  
AGCCAGCGGC GCCTGCGCCG GTGCCACAA TCGTGCGCGA ACAAACTAA CCAGAACA 3720  
TCATACCGGC GGCACCGCCG CCACCACCTT CTCCTGTGCC TAACATTCCA GCGCCTCCAC 3780  
CACTACCACC ACCATCGATG TCTGAATTGC CGCCCGCTCC ACCAATGCCG ACGGAACCTC 3840  
AACCCGCTGC ACCTTTAGAC GACAGACAAC AATTGTTGGA AGCTATTAGA AACGAAAAA 3900  
ATCGCACTCG TCTCAGACCG GCTCTCTTAA GGATAGCTCA ACCAAAAACG GCGCCCCGAA 3960

## VVU 70/41043

15/75

# FIG. 7H

CCTGCTGGGG AGCCTGGGGA CTTTCCACAC CCTAACTGAC ACACATTCCA CAGAAATTAAT 4680  
TCCCCTAGTT ATTAATAGTA ATCAATTACG GGGTCATTAG TTCATAGCCC ATATATGGAG 4740  
TTCCGCGTTA CATAACTTAC GGTAAATGGC CCGCCTGGCT GACCGCTCAA CGACCCCCCGC 4800  
CCATTGACGT CAATAATGAC GTATGTTCCTC ATAGTAACGC CAATAGGGAC TTTCCATTGA 4860  
CGTCAATGGG TGGACTATTT ACGGTAACT GCCCACTTGG CAGTACATCA AGTGTATCAT 4920  
ATGCCAAGTA CGCCCCCTAT TGACGTCAAT GACGGTAAAT GGCCCCGCCTG GCATTATGCC 4980  
CAGTACATGA CCTTATGGGA CTTTCCTACT TGGCAGTACA TCTACGTATT AGTCATCGCT 5040  
ATTACCATGG TGATGCGGTT TTGGCAGTAC ATCAATGGGC GTGGATAGCG GTTTGACTCA 5100  
CGGGGATTTC CAAGTCTCCA CCCCATTGAC GTCAATGGGA GTTTGTTTTG AAGCTTGGCC 5160  
GGCCATATAA ACGGCGGGCCA GCTTTATTTA ACGTGTTTAC GTCGAGTCAA TTGTACACTA 5220  
ACGACAGTGA TGAAAGAAAT ACAAAGCGC ATAATATTTT GAACGACGTC GAACCTTTAT 5280

# FIG. 71

TACAAAACAA AACACAAACG AATATCGACA AAGCTAGATT GCTGCTACAA GATTTGGCAA 5340  
GTTTGTGGC GTTGAGCGAA AATCCATTAG ATAGTCCAGC CATCGGTTCCG GAAAAACAAC 5400  
CCTTGTTTGA AACTAATCGA AACCTATTTT ACAAATCTAT TGAGGATTTA ATATTTAAT 5460  
TCAGATATAA AGACGCTGAA AATCATTTGA TTTTCGCTCT AACATACCAC CCTAAAGATT 5520  
ATAAATTIAA TGAATTATTA AAATACATCA GCAACTATAT ATTGATAGAC ATTCCAGTT 5580  
TGTGATATTA GTTTGTGCGT CTCATTACAA TGGCTGTTAT TTTTAACAAC AAACAACCTGC 5640  
TCGCAGACAA TAGTATAGAA AAGGGAGGTG AACTGTTTTT GTTTAACGGT TCGTACAAACA 5700  
TTTTGGAAAG TTATGTTAAT CCGGTGCTGC TAAAAAATGG TGTAATTGAA CTAGAAGAAG 5760  
CTGCGTACTA TGCCGGCAAC ATATTGTACA AAACCGACGA TCCCAAATTC ATTGATTATA 5820  
TAAATTTAAT AATTAAAGCA ACACACTCCG AAGAACTACC AGAAAATAGC ACTGTTGTAA 5880  
ATTACAGAAA AACTATGCGC AGCGGTACTA TACACCCCAT TAAAAAAGAC ATATATATTT 5940

# FIG. 7J

ATGACAACAA AAAATTTACT CTATACGATA GATACATATA TGGATACGAT AATAACTATG 6000  
TTAATTTTAA TGAGGAGAAA AATGAAAAAG AGAAGGAATA CGAAGAAGAA GACGACAAGG 6060  
CGTCTAGTTT ATGTGAAAAT AAAATTATAT TGTCGCAAAT TAACTGTGAA TCATTTGAAA 6120  
ATGATTTTAA ATATTACCTC AGCGATTATA ACTACGCGTT TTCAATTATA GATAACACTA 6180  
CAAATGTTCT TGTTCGCGTTT GGTTTGATC GTTAATAAAA AACAAATTTA GCATTTATAA 6240  
TTGTTTTATT ATTCATAAT TACAAATAGG ATTGAGACCC TTGCAGTTGC CAGCAAACGG 6300  
ACAGAGCTTG TCGAGGAGAG TTGTGATTC ATTGTTGCC TCCCTGCTGC GGTTTTTGAC 6360  
CGAAGTTCAT GCCAGTCCAG CGTTTTTGCA GCAGAAAAGC CGCCGACTTC GGTTTGCGGT 6420  
CGCGAGTGAA GATCCCTTTC TTGTTACCGC CAACGGCGCA TATGCCCTTG CAGGTCGCAA 6480  
AATCGGCGAA ATTCCATACC TGTTACCCGA CGACGGCGCT GACGCGATCA AAGACGCGGT 6540  
GATACATATC CAGCCATGCA CACTGATACT CTTCACTCCA CATGTCGGTG TACATTGAGT 6600

# FIG. 7K

GCAGCCCGGC TAACGTATCC ACGCCGTATT CGGTGATGAT AATCGGCTGA TGCAGTTTCT 6660  
CCTGCCAGGC CAGAAGTTCT TTTTCCAGTA CCTTCTCTGC CGTTTCCAAA TCGCCGCTTT 6720  
GGACATACCA TCCGTAATAA CGGTTCAGGC ACAGCACATC AAAGAGATCG CTGATGGTAT 6780  
CGGTGTGAGC GTCGCAGAAC ATTACATTGA CGCAGGTGAT CGGACGCGTC GGGTCGAGTT 6840  
TACGCGTTGC TTCCGCCAGT GCGCGGAAAT ATTCCCGTGC ACCTTGCGGA CGGGTATCCG 6900  
GTTTCGTTGC AATACTCCAC ATCACCACGC TTGGGTGGT TTTGTACGC GCTATCAGCT 6960  
CTTTAATCGC CTGTAAGTGC GCTTGGTGAG TTTCCCCGTT GACTGCCCTCT TCGTTGTACA 7020  
GTTCTTTCGG CTTGTTGCC GCTTCGAAAC CAATGCCCTAA AGAGAGGTTA AAGCCGACAG 7080  
CAGCAGTTTC ATCAATCACC ACGATGCCAT GTTCATCTGC CCAGTCGAGC ATCTCTTCAG 7140  
CGTAAGGGTA ATGCGAGGTA CGGTAGGAGT TGGCCCTAAT CCAGTCCATT AATGCGTGGT 7200  
CGTGACCAT CAGCACGTTA TCGAATCCTT TGCCACGCAA GTCCGCATCT TCATGACGAC 7260

# FIG. 7L

CAAAGCCAGT AAAGTAGAAC GGTTTGTGGT TAATCAGGAA CTGTTGCCCC TTCACTGCCA 7320  
CTGACCGGAT GCCGACGCGA AGCGGGTAGA TATCACACTC TGTCTGGCTT TTGGCTGTGA 7380  
CGCACAGTTC ATAGAGATAA CCTTCACCCG GTTGCCAGAG GTGCGGATTC ACCACTTGCA 7440  
AAGTCCCGCT AGTGCCTTGT CCAGTTGCAA CCACCTGTTG ATCCGCATCA CGCAGTTCAA 7500  
CGCTGACATC ACCATTGGCC ACCACCTGCC AGTCAACAGA CGCGTGGTTA CAGTCTTGCG 7560  
CGACATGCGT CACTACGGTG ATATCGTCCA CCCAGGTGTT CGGCGTGGTG TAGAGCATT 7620  
CGCTGCGATG GATTCCGGCA TAGTTAAAGA AATCATGGAA GTAAGATTGC TTTTCTTGC 7680  
CGTTTTCGTT GGTAATCACC ATTCCCGGCG GGATAGTCTG CCAGTTCAGT TCGTTGTTCA 7740  
CACAAACGGT GATACCCCTC GACGGATTAA AGACTTCAAG CGGTCAACTA TGAAGAAGTG 7800  
TTCGTCITCG TCCCAGTAAG CTATGTCTCT AGAATGTAGC CATCCATCCT TGTC AATCAA 7860  
GGCGTTGGTC GCTTCCGGAT TGTTACATA ACCGGACATA ATCATAGGTC CTC TGACACA 7920

# FIG. 7M

TAATACGCCT CTC TGATTAA CGCC CAGCGT TTTCCCGGTA TCCAGATCCA CAACCTTCGC 7980  
TTCAAAAAAT GGAACAAC TT TACCGACCGC GCCCGGTTTA TCATCCCCCT CGGGTGTAAT 8040  
CAGATAGCT GATGTAGTCT CAGTGAGCCC ATATCCTTGT CGTATCCCTG GAAGATGGAA 8100  
GCGTTTTGCA ACCGCTTCCC CGACTTCTTT CGAAAGAGGT GCGCCCCCAG AAGCAATTTC 8160  
GTGTAAATTA GATAAATCGT ATTTGTCAAT CAGAGTGCTT TTGGCGAAGA ATGAAAATAG 8220  
GGTTGGTACT AGCAACGCAC TTTGAATTTT GTAATCCTGA AGGGATCGTA AAAACAGCTC 8280  
TTCTTCAAAT CTATACATTA AGACGACTCG AAATCTACAT ATCAAATATC CGAGTGTAGT 8340  
AAACATTCCA AAACCGTGAT GGAATGGAAC AACACTTAA ATCGCAGTAT CCGGAATGAT 8400  
TTGATTGCCA AAAATAGGAT CTCTGGCATG CGAGAATCTA GCGCAGGCAG TTCTATGCCG 8460  
AAGGGCCACA CCCTTAGGTA ACCCAGTAGA TCCAGAGGAA TTGTTTTGTC ACGATCAAAG 8520  
GACTCTGGTA CAAAATCGTA TTCATTAAAA CCGGGAGGTA GATGAGATGT GACGAAAGGTG 8580

# FIG. 7N

TACATCGACT GAAATCCCTG GTAATCCGTT TTAGAATCCA TGATAATAAT TTTCTGGATT 8640  
ATTGGTAATT TTTTITGAC GTTCAAAATT TTTTGCAACC CCTTTTITGGA AACAAACACT 8700  
ACGGTAGGCT GCGAAATGTT CATACTGTTG AGCAATTTCAC GTTCATTATA AATGTCGTTT 8760  
GCGGGCGCAA CTGCAACTCC GATAAATAAC GCGCCCAACA CCGGCATAAA GAATTGAAGA 8820  
GAGTTTTTAC TGCATACGAC GATTCTGTGA TTTGTATTCA GCCCATATCG TTTTCATAGCT 8880  
TCTGCCAACC GAACGGACAT TTCGAAGTAT TCCGCGTACG TGATGTTTAC CTCGATATGT 8940  
GCATCTGTAA AAGGAATTGT TCCAGGAACC AGGGCGTATC TCTTCATAGC CTTATGCAGT 9000  
TGCTCTCCAG CCGTTCCATT CTCTAGCTTT GCCTCTCAAT TTCCTTATTG CATAATGAGA 9060  
AAAAAAGGAA AATTAATTTT AACACCAATT CAGTAGTTGA TTGAGCAAAT GCGTTGCCAA 9120  
AAAGGATGCT TTAGAGACAG TGTTCTCTGC ACAGATAAGG ACAAACATCA TTCAGAGGGA 9180  
GTACCCAGAG CTGAGACTCC TAAGCCAGTG AGTGGCACAG CATTCTAGGG AGAAATATGC 9240

# FIG. 7P

TTGTCATCAC CGAAGCCTGA TTCCGTAGAG CCACACCTTG GTAAGGGCCA ATCTGCTCAC 9300  
ACAGGATAGA GAGGGCAGGA GCCAGGGCAG AGCATATAAG GTGAGGTAGG ATCAGTTGCT 9360  
CCTCACATT GCTTCTGACA TAGTTGTGTT GGGAGCTTGG ATCGATCCAC CATGGGCTTC 9420  
AATACCCCTGA TTGACTGGAA CAGCTGTAGC CCTGAACAGC AGCGTGCGCT GCTGACGCGT 9480  
CCGGCGATT CCGCCTCTGA CAGTATTACC CGGACGGTCA GCGATATTCT GGATAATGCA 9540  
AAAACGCGCG GTGACGATGC CCTGCGTGAA TACAGCGCTA AATTTGATAA AACAGAAGTG 9600  
ACAGCGCTAC GCGTCACCCC TGAAGAGATC GCCGCCGCCG GCGCGCGTCT GAGCGACGAA 9660  
TTAAACAGG CGATGACCGC TGCCGTCAA AATATTGAAA CGTTCCATTG CGCGCAGACG 9720  
CTACCGCTTG TAGATGTGGA AACCCAGCCA GCGTGCGGTT GCCAGCAGGT TACGCGTCCC 9780  
GTCCTGCTG TCGGTCTGTA TATTCGCCGC GGCTCGGCTC CGCTCTTCTC AACGGTGCTG 9840  
ATGCTGGCGA CGCCGGCGCG CATTGCGGGA TGCTAGAAGG TGGTTCTGTG CTCGCCGCCG 9900

# FIG. 7Q

CCCATCGCTG ATGAAATCCT CTATGCGGCG CAACTGTGTG GCGTGCAGGA ATTCTTTAAC 9960  
CTCGGCGGCG CGCAGGCGAT TGCCGCTCTG GCCTTCGGCA GCGAGTCCGT ACCGAAAGTG 10020  
GATAAAATTT TTGGCCCCGG CAACGCCCTT GTAACCGAAG CCAAACGTCA GGTCAGCCAG 10080  
CGTCTCGACG GCGCGGGCTAT CGATATGCCA GCCGAGCCGT CTGAAGTACT GGTGATCGCA 10140  
GACAGCGGCG CAACACCGGA TTTCGTCGCT TCTGACCTGC TCTCCCAGAC TGAGCACGGC 10200  
CCGGATTCCC AGGTGATCCT GCTGACGCCT GATGCTGACA TTGCCCGCAA GGTGGCGGAG 10260  
GCGGTAGAAC GTCAACTGGC GGAAGTGGC GCGCGGACA CCGCCTGGCA GGCCCTGAGC 10320  
GCCAGTCGTC TGATTGTGAC CAAAGATTGA GCGCAGTGCG TCGCCATCTC TAATCAGTAT 10380  
GGGCCGGAAC ACTTAATCAT CCAGACGCGC AATGCGCGCG ATTTGGTGGA TCGGATTACC 10440  
AGCGCAGGCT CCGTATTTCT CCGCGACTGG TCGCCGGAAT CCGCCGGTGA TTACGCTTCC 10500  
GGAACCAACC ATGTTTTACC GACCTATGGC CATACTGCTA CCTGTTCAG CCTTGGGTTA 10560

# FIG. 7R

GCGGATTTCC AGAAACGGAT GACCGTTTCAG GAACTGTCTGA AAGCGGGCTT TTCCGCTCTG 10620  
GCATCAACCA TTGAAACATT GCGGGGGGCA GAACGTCTGA CCGCCCATAA AAATGCCGTG 10680  
ACCCTGCGCG TAAACGCCCT CAAGGAGCAA GCATGAGCAC TGAAACACT CTCAGCGTCG 10740  
CTGACTTAGC CCGTGAAAT GTCCGCAACC TGGAGATCCA GACATGATAA GATACATTGA 10800  
TGAGTTTGGA CAAACCACAA CTAGAATGCA GTGAAAAAA TGCTTTATTT GTGAAATTTG 10860  
TGATGCTATT GCTTTATTTG TAACCATTAT AAGCTGCAAT AAACAAGTTA ACAACAACAA 10920  
TTGCATTCAT TTTATGTTTC AGGTTCAGGG GGAGGTGTGG GAGGTTTTTT AAAGCAAGTA 10980  
AAACCTCTAC AAATGTGGTA TGGCTGATTA TGATCTCTAG CTCGACGGGG CGCCTGGCCG 11040  
CTACTAACTC TCTCCTCCCT CCTTTTCCCT GCAGGCTCAA GCGCGCATG CCCGACGGCG 11100  
AGGATCTCGT CGTGACCCAT GGCGATGCCT GCTTGCCGAA TATCATGGTG GAAAATGGCC 11160  
GCTTTTCTGG ATTCATCGAC TGTGGCCGGC TGGGTGTGGC GGACCGCTAT CAGGACATAG 11220

# FIG. 7S

CGTTGGCTAC CCGTGATATT GCTGAAGAGC TTGGCGGCCG ATGGGCTGAC CGCTTCCTCG 11280  
TGCTTTACGG TATCGCCGCT CCCGATTCCG AGCGCATCGC CTTCTATCGC CTTCTTGACG 11340  
AGTTCTTCTG AGCGGGACTC TGGGGTTCTG AATGACCGAC CAAGCGACGC CCAACCTGCC 11400  
ATCACGAGAT TTCGATTCCA CCGCCGCCTT CTATGAAAGG TTGGGCTTCG GAATCGTTTT 11460  
CCGGGACGCC GGCTGGATGA TCCTCCAGCG CGGGGATCTC ATGCTGGAGT TCTTCGCCCA 11520  
CCCCAACTTG TTTATTGCAG CTTATAATGG TTACAAATAA AGCAATAGCA TCACAAATTT 11580  
CACAAATAAA GCATTTTTTT CACTGCATTC TAGTTGTGGT TTGTCCAAAC TCATCAATCT 11640  
ATCTTATCAT GTCTGGATCG CGGCCGGTCT CTCTCTAGCC CTAGGTCTAG ACTTGGCAGA 11700  
ACATATCCAT CGCGTCCGCC ATCTCCAGCA GCCGCACGCG GCGCATCTCG GGCAGCGTTG 11760  
GGTCCTGGCC ACGGGTGCGC ATGATCGTGC TCCTGTCTGT GAGGACCCGG CTAGGCTGGC 11820  
GGGGTTGCCT TACTGGTTAG CAGAAATGAAT CACCGATACG CGAGCGAACG TGAAGCGACT 11880

# FIG. 7T

GCTGCTGCAA AACGTCTGCG ACCTGAGCAA CAACATGAAT GGTCTTCGGT TTCCGTGTTT  
11940  
CGTAAAGTCT GGAACGCGG AAGTCAGCGC CCTGCACCAT TATGTTCCGG ATCTGCATCG  
12000  
CAGGATGCTG CTGGCTACCC TGTGGAACAC CTACATCTGT ATTAACGAAG CGCTGGCATT  
12060  
GACCCTGAGT GATTTTCTC TGGTCCCGCC GCATCCATAC CGCCAGTTGT TTACCCCTCAC  
12120  
AACGTTCCAG TAACCGGGCA TGTTTCATCAT CAGTAACCCG TATCGTGAGC ATCCTCTCTC  
12180  
GTTTCATCGG TATCATTACC CCCATGAACA GAAATCCCCC TTACACGGAG GCATCAGTGA  
12240  
CCAAACAGGA AAAAACCGCC CTTAACATGG CCCGCTTTAT CAGAAGCCAG ACATTAACGC  
12300  
TTCTGGAGAA ACTCAACGAG CTGGACGCGG ATGAACAGGC AGACATCTGT GAATCGCTTC  
12360  
ACGACCACGC TGATGAGCTT TACCGCAGCT GCCTCGCGCG TTTCGGTGAT GACGGTGAAA  
12420  
ACCTCTGACA CATGCAGCTC CCGGAGACGG TCACAGCTTG TCTGTAAGCG GATGCCGGGA  
12480  
GCAGACAAGC CCGTCAGGGC GCGTCAGCGG GTGTTGGCGG GTGTCGGGGC GCAGCCATGA  
12540

27/75

# FIG. 7U

CCCAGTCACG TAGCGATAGC GGAGTGTATA CTGGCTTAAC TATGCGGCAT CAGAGCAGAT 12600  
TGTA CTGAGA GTGCACCATATA TCGGGTGTGA AATACCGCAC AGATGCGTAA GGAGAAAATA 12660  
CCGCATCAGG CGCTCTTCCG CTTCCTCGCT CACTGACTCG CTGCGCTCGG TCGTTCGGCT 12720  
GCGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAATACGG TTATCCACAG AATCAGGGGA 12780  
TAACGCAGGA AAGAACATGT GAGCAAAGG CCAGCAAAG GCCAGGAACC GTAAAAGGC 12840  
CGCGTTGCTG GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG 12900  
CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAGA TACCAGGCGT TTCCCCCTGG 12960  
AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT ACCGGATACC TGTCCGCTT 13020  
TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTTCGGT 13080  
GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCCGTTTCAGC CCGACCGCTG 13140  
CGCCTTATCC GGTAACATC GTC TTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT 13200

# FIG. 7V

GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT 13260  
CTTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGGACA GTATTGGTA TCTGCGCTCT 13320  
GCTGAAGCCA GTTACCCTTCG GAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC 13380  
CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC 13440  
TCAAGAAGAT CCTTTGATCT TTCTACGGG GTCTGACGCT CAGTGGAACG AAAACTCACG 13500  
TTAAGGGATT TTGGTCATGA GATTATCAA AAGGATCTTC ACCTAGATCC TTTTAAATTA 13560  
AAAATGAAGT TTAAATCAA TCTAAAGTAT ATATGAGTAA ACTTGGTCTG ACAGTTACCA 13620  
ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA TTTCGTTTCAAT CCATAGTTGC 13680  
CTGACTCCCC GTCGTGTAGA TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC 13740  
TGCAATGATA CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC 13800  
AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCTCCA TCCAGTCTAT 13860

# FIG. 7W

TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT AATAGTTTGC GCAACGTTGT 13920  
TGCCATTGCT GCAGGCATCG TGGTGTACAG CTCGTCGTTT GGTATGGCTT CATTACAGCTC 13980  
CGGTTCCCAA CGATCAAGGC GAGTIACATG ATCCCCCATG TTGTGC AAAA AAGCGGTTAG 14040  
CTCCTTCGGT CCTCCGATCG TTGTCAGAAG TAAGTGGCC GCAGTGTTAT CACTCATGGT 14100  
TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC GTAAGATGCT TTTCTGTGAC 14160  
TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGATG CGGCGACCGA GTTGCTCTTG 14220  
CCCGGCGTCA ACACGGGATA ATACCGCGCC ACATAGCAGA ACTTTAAAAG TGCTCATCAT 14280  
TGGAAAACGT TC TTCGGGGC GAAAACTCTC AAGGATCTTA CCGCTGTTGA GATCCAGTTC 14340  
GATGTAACCC ACTCGTGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC 14400  
TGGGTGAGCA AAACAGGAA GGCAAAATGC CGCAAAAAG GGAATAAGG CGACACGGAA 14460  
ATGTTGAATA CTCATACTCT TCCTTTTTCA ATATTATTGA AGCATTTATC AGGGTTATTG 14520

# FIG. 7X

TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG  
14580  
CACATTTCCC CGAAAAGTGC CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAAC  
14640  
CTATAAAAT AGGCGTATCA CGAGGCCCTT TCGTCTTCAA GAA  
14683

# FIG. 8A

TTAATTAAGG GGC GGAGAAAT GGGCGGAACT GGGCGGAGTT AGGGCGGGGA TGGGCGGAGT 60  
TAGGGCGGG ACTATGGTTG CTGACTAATT GAGATGCATG CTTTGCATAC TTCTGCCTGC 120  
TGGGAGCCT GGGGACTTTC CACACCTGGT TGCTGACTAA TTGAGATGCA TGCTTTTGCAT 180  
ACTTCTGCCT GCTGGGGAGC CTGGGGACTT TCCACACCCCT AACTGACACA CATTCCACAG 240  
AATTAATTCC CCTAGTTATT AATAGTAATC AATTACGGGG TCATTAGGTC ATAGCCCAT 300  
TATGGAGTTC CGCGTTACAT AACTTACGGT AAATGGCCCCG CCTGGCTGAC CGCCCCAACGA 360  
CCCCGCCCCA TTGACGTCAA TAATGACGTA TGTTC CCATA GTAACGCCAA TAGGGACTTT 420  
CCATTGACGT CAATGGGTGG ACTATTTACG GTAACTGCC CACTTGGCAG TACATCAAGT 480  
GTATCATATG CCAAGTACGC CCCCTATTGA CGTCAATGAC GGTAATGGC CCGCCTGGCA 540  
TTATGCCCCAG TACATGACCT TATGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT 600  
CATCGCTATT ACCATGGTGA TCGGGTTTTG GCAGTACATC AATGGCGGTG GATAGCGGTT 660  
TGA CTCACGG GGATTTCCAA GTCTCCACCC CATTGACGTC AATGGGAGTT TGT TTTGAAG 720  
CTTGGCCGGC CATATAAACG GCGGCCAGCT TTATTTAACG TGT TTAGCTC GAGTCAATTG 780  
TACACTAACG ACAGTGATGA AAGAAATACA AAAGCGCATA ATATTTTGAA CGACGTCGAA 840

32/75

# FIG. 8B

CCTTTATTAC AAAACAAAAC ACAACGAAT ATCGACAAAG CTAGATTGCT GCTACAAGAT 900  
TTGGCAAGTT TTGTGGCGTT GAGCGAAAAT CCATTAGATA GTCCAGCCAT CGGTTCCGAA 960  
AAACAACCCCT TGTTTGAAAC TAATCGAAAC CTATTTTACA AATCTATTGA GGATTTAATA 1020  
TTTAAATTCA GATATAAAGA CGCTGAAAAT CATTTGATTT TCGCTCTAAC ATACCACCCCT 1080  
AAAGATTATA AATTTAATGA ATTATTAAA TACATCAGCA ACTATATATT GATAGACATT 1140  
TCCAGTTTGT GATATTAGTT TGTGCGTCTC ATTACAATGG CTGTTATTTT TAACAACAAA 1200  
CAACTGCTCG CAGACAATAG TATAGAAAAG GGAGGTGAAC TGTTTTTGTT TAACGGTTCTG 1260  
TACAACATTT TGGAAAGTTA TGTTAATCCG GTGCTGCTAA AAAATGGTGT AATTGAACTA 1320  
GAAGAAGCTG CGTACTATGC CGGCAACATA TTGTACAAA CCGACGATCC CAAATTCATT 1380  
GATTATATA ATTTAATAAT TAAAGCAACA CACTCCGAAG AACTACCAGA AAATAGCACT 1440  
GTTGTAAATT ACAGAAAAAC TATGCGCAGC GGTA CTATAC ACCCCATTAA AAAAGACATA 1500  
TATATTTATG ACAACAAAAA ATTTACTCTA TACGATAGAT ACATATATGG ATACGATAAT 1560  
AACTATGTTA ATTTTATGA GGAGAAAAAT GAAAAAGAGA AGGAATACGA AGAAGAAGAC 1620  
GACAAGGCGT CTAGTTTATG TGAAAAATAA ATTATATTGT CGCAAATTAA CTGTGAATCA 1680

# FIG. 8C

TTTGAAAATG ATTTAAATA TTACCTCAGC GATTATACT ACGCGTTTTC AATTATAGAT 1740  
AATACTACAA ATGTTCTTGT TCGGTTTGGT TTGTATCGTT AATAAAAAAC AAATTTAGCA 1800  
TTTATAATTG TTTTATTATT CAATAATTAC AAATAGGATT GAGACCCCTTG CAGTTGCCAG 1860  
CAAACGGACA GAGCTTGTCG AGGAGAGTTG TTGATTTCATT GTTTGCCCTCC CTGCTGCGGT 1920  
TTTTCACCGA AGTTCATGCC AGTCCAGCGT TTTTGCAGCA GAAAAGCCGC CGACTTCGGT 1980  
TTGCGGTGCG GAGTGAAGAT CCCTTTCTTG TTACCGCCAA CGCGCAATAT GCCTTGCGAG 2040  
GTCGCAAAAT CGGCGAAATT CCATACCTGT TCACCGACGA CGGCGCTGAC GCGATCAAAG 2100  
ACGCGGTGAT ACATATCCAG CCATGCACAC TGATACTCTT CACTCCACAT GTCGGTGTAC 2160  
ATTGAGTGCA GCCCGGCTAA CGTATCCACG CCGTATTTCGG TGATGATAAT CGGCTGATGC 2220  
AGTTTCTCCT GCCAGGCCAG AAGTTCTTTT TCCAGTACCT TCTCTGCCGT TTCCAAATCG 2280  
CCGCTTTGGA CATACCATCC GTAATAACGG TTCAGGCACA GCACATCAA GAGATCGCTG 2340  
ATGGTATCGG TGTGAGCGTC GCAGAACATT ACATTGACGC AGGTGATCGG ACGCGTCGGG 2400  
TCGAGTTTAC GCGTTGCTTC CGCCAGTGGC GCGAAATATT CCCGTGCACC TTGCGGACGG 2460  
GTATCCGGTT CGTTGGCAAT ACTCCACATC ACCACGCTTG GGTGGTTTTT GTCACGCGCT 2520

# FIG. 8D

ATCAGCTCTT TAATCGCCTG TAAGTGGCT TGCTGAGTTT CCCC GTTGAC TGCCTCTTCG 2580  
CTGTACAGTT CTTTCGGCTT GTTGCCCGCT TCGAAACCAA TGCCTAAAGA GAGGTTAAAG 2640  
CCGACAGCAG CAGTTTCATC AATCACCACG ATGCCATGTT CATCTGCCCA GTCGAGCATC 2700  
TCTTCAGCGT AAGGTAATG CGAGGTACGG TAGGAGTTGG CCCC AATCCA GTCCATTAAAT 2760  
GCGTGGTCGT GCACCATCAG CACGTTATCG AATCCTTTGC CACGCAAGTC CGCATCTTCA 2820  
TGACGACCAA AGCCAGTAA GTAGAACGGT TTGTGGTTAA TCAGGAACTG TTCGCCCTTC 2880  
ACTGCCACTG ACCGGATGCC GACGCGAAGC GGTAGATAT CACACTCTGT CTGGCTTTTG 2940  
GCTGTGACGC ACAGTTCATA GAGATAACCT TCACCCGGTT GCCAGAGGTG CGGATTCAAC 3000  
ACTTGCAAAG TCCCGCTAGT GCCTTGTCCA GTTGCAACCA CCTGTTGATC CGCATCACGC 3060  
AGTTCAACGC TGACATCACC ATTGGCCACC ACCTGCCAGT CAACAGACGC GTGGTTACAG 3120  
TCTTGCGCGA CATGCGTCAC CACGGTGATA TCGTCCACCC AGGTGTTCCG CGTGGTGTAG 3180  
AGCATTACGC TCGGATGGAT TCCGGCATAG TTAAAGAAAT CATGGAAGTA AGACTGCTTT 3240  
TTCTTGCCGT TTTCGTCCGT AATCACCATT CCCGGCGGGA TAGTCTGCCA GTTCAGTTTCG 3300  
TTGTTACAC AAACGGTGAT ACCCCTCGAC GGATTAAGA CTTCAAGCGG TCAACTATGA 3360

35/75

# FIG. 8E

AGAAGTGTTT GTCTTCGTCC CAGTAAGCTA TGCTCCAGA ATGTAGCCAT CCATCCTTGT 3420  
CAATCAAGGC GTTGGTCGCT TCCGGATTGT TTACATAACC GGACATAATC ATAGGTCCTC 3480  
TGACACATAA TTCGCCCTCTC TGATTAAAGC CCAGCGTTTT CCCGGTATCC AGATCCACAA 3540  
CCTTCGCTTC AAAAATGGA ACAACTTTAC CGACCGCGCC CGGTTTATCA TCCCCCTCGG 3600  
GTGTAATCAG AATAGCTGAT GTAGTCTCAG TGAGCCCCATA TCCTTGTCGT ATCCCTGGAA 3660  
GATGGAAGCG TTTTGCAACC GCTTCCCCGA CTTCTTTTGA AAGAGGTGCG CCCCCAGAAG 3720  
CAATTCGTG TAAATTAGAT AAATCGTATT TGTCATCAG AGTGCTTTTG GCGAAGAATG 3780  
AAAATAGGGT TGGTACTAGC AACGCACCTT GAATTTTGTG ATCCTGAAGG GATCGTAAAA 3840  
ACAGCTCTTC TTCAAATCTA TACATTAAGA CGACTCGAAA TCCACATATC AAATATCCGA 3900  
GTGTAGTAA CATTCCAAA CCGTGATGGA ATGGAACAAC ACTTAAATC GCAGTATCCG 3960  
GAATGATTG ATTGCCAAA ATAGGATCTC TGGCATGCCG GAATCTAGCG CAGGCAGTTC 4020  
TATCGGGAAG GGCCACACCC TTAGGTAACC CAGTAGATCC AGAGGAATTG TTTGTGACG 4080  
ATCAAAGGAC TCTGGTACAA AATCGTATTC ATTAAACCG GGAGGTAGAT GAGATGTGAC 4140  
GAACGTGTAC ATCGACTGAA ATCCCTGGTA ATCCGTTTTA GAATCCATGA TAATAATTTT 4200

36/75

# FIG. 8F

CTGGATTATT GGTAATTTTT TTTCACGTT CAAAATTTTT TGCAACCCCT TTTTGGAAC 4260  
AAACACTACG GTAGGCTGCG AAATGTTTCACT ACTGTTGAGC AATTCACGTT CATTATAAAT 4320  
GTCGTTGCGG GCGCAACTG CAACTCCGAT AAATAACGCG CCCAACACCG GCATAAAGAA 4380  
TTGAAGAGAG TTTTCACTGC ATACGACGAT TCTGTGATTT GTATTCAGCC CATATCGTTT 4440  
CATAGCTTCT GCCAACCAGAA CGGACATTTT GAAGTATTCC GCGTACGTGA TGTTACACCTC 4500  
GATATGTGCA TCTGTAAAAG GAATTGTTCC AGGAACCAGG GCGTATCTCT TCATAGCCCTT 4560  
ATGCAGTTGC TCTCCAGCGG TTCCATCCTC TAGCTTTGCT TCTCAATTTT TTTATTTGCAT 4620  
AATGAGAAA AAAGGAAAAT TAATTTTAAC ACCAATTCAG TAGTTGATTG AGCAAATGCG 4680  
TTGCCAAAA GGATGCTTTA GAGACAGTGT TCTCTGCACA GATAAGGACA AACATTATTC 4740  
AGAGGGAGTA CCCAGAGCTG AGACTCCTAA GCCAGTGAGT GGCACAGCAT TCTAGGGAGA 4800  
AATATGCTTG TCATCACCGA AGCCTGATTC CGTAGAGCCA CACCTTGTA AGGGCCAATC 4860  
TGCTCACACA GGATAGAGAG GGCAGGAGCC AGGGCAGAGC ATATAAGGTG AGGTAGGATC 4920  
AGTTGCTCCT CACATTTGCT TCTGACATAG TTGTGTTGGG AGCTTGGATC GATCCACCAT 4980  
GGGCTTCAAT ACCCTGATTG ACTGGAACAG CTGTAGCCCT GAACAGCAGC GTGCGCTGCT 5040

3775

# FIG. 8G

GACGCGTCCG GCGATTTCGG CCTCTGACAG TATTACCCGG ACGGTCAGCG ATATTCTGGA 5100  
TAATGTAAA ACGCGCGGTG ACGATGCCCT GCGTGAATAC AGCGCTAAAT TTGATAAAAC 5160  
AGAAGTGACA GCGCTACGGG TCACCCCTGA AGAGATCGCC GCCGCCGGCG CGCGTCTGAG 5220  
CGACGAATTA AACAGGGGA TGACCGCTGC CGTCAAAAAT ATTGAAACGT TCCATTCCGC 5280  
GCAGACGCTA CCGCCTGTAG ATGTGGAAC CCAGCCAGGC GTGCGTTGCC AGCAGGTTAC 5340  
GCGTCCCCTC TCGTCTGTGC GTCTGTATAT TCCC GGCGGC TCGGCTCCGC TCTTCTCAAC 5400  
GGTGTGATG CTGGCGACGC CGCGCGCAT TCGGGGATGC CAGAAGGTGG TTCTGTGCTC 5460  
GCCGCCGCC ATCGCTGATG AAATCCTCTA TCGGGCGCAA CTGTGTGGCG TGCAGGAAAT 5520  
CTTTAACGTC GCGGGCGCG AGGCGATTGC CGCTCTGGCC TTCGGCAGCG AGTCCGTACC 5580  
GAAAGTGGAT AAAATTTTGG GCCCGGGCAA CGCCTTTGTA ACCGAAGCCA AACGTCAGGT 5640  
CAGCCAGCGT CTCGACGGCG CGGCTATCGA TATGCCAGCC GGGCCGTCTG AAGTACTGGT 5700  
GATCGCAGAC AGCGGGCGAA CACCGGATTT CGTCGCTTCT GACCTGCTCT CCCAGGCTGA 5760  
GCACGGCCCG GATTCCCAGG TGATCCTGCT GACGCCTGAT GCTGACATTG CCCGCAAGGT 5820  
GGCGAGGCG GTAGAACGTC AACTGGCGGA ACTGCCGCG GCGGACACCG CCCGGCAGGC 5880

# FIG. 8H

CCTGAGCGCC AGTCGTCTGA TTGTGACCAA AGATTAGCG CAGTGCCTCG CCATCTCTAA 5940  
TCAGTATGGG CCGGAACACT TAATCATCCA GACGCGCAAT GCGCGCGATT TGGTGGATGC 6000  
GATTACCAGC GCAGGCTCGG TATTTCTCGG CGACTGGTGG CCGGAATCCG CCGGTGATTA 6060  
CGCTTCCGGA ACCAACCATG TTTTACCGAC CTATGGCTAT ACTGCTACCT GTTCCAGCCT 6120  
TGGGTTAGCG GATTTCCAGA AACGGATGAC CGTTCAGGAA CTGTGGAAG CGGGCTTTTC 6180  
CGCTCTGGCA TCAACCAATTG AAACATTGGC GCGGCGAGAA CGTCTGACCG CCCATAAAAA 6240  
TGCCGTGACC CTGCGCGTAA ACGCCCTCAA GGAGCAAGCA TGAGCACTGA AAACACTCTC 6300  
AGCGTCGCTG ACTTAGCCCG TGAAAATGTC CGCAACCTGG AGATCCAGAC ATGATAAGAT 6360  
ACATTGATGA GTTTGGACAA ACCACAACCTA GAATGCAGTG AAAAAAATGC TTTATTTGTG 6420  
AAATTTGTGA TGCTATTGCT TTATTTGTAA CCATTATAAG CTGCAATAAA CAAGTTAACA 6480  
ACAACAATTG CATTCAATTT ATGTTTCAGG TTCAGGGGGA GGTGTGGGAG GTTTTTTAAA 6540  
GCAAGTAAA CCTCTACAAA TGTGGTATGG CTGATTATGA TCTCTAGCTC GACGGCGCGC 6600  
CTCTAGAGCA GTGTGGTTTT GCAAGAGGAA GCAAAAAGCC TCTCCACCCA GGCCTGGAAT 6660  
GTTTCCACCC AATGTCGAGC AGTGTGGTTT TGCAAGAGGA AGCAAAAAGC CTCTCCACCC 6720

# FIG. 8I

AGGCCTGGAA TGTTTCCACC CAATGTCGAG CAAACCCCGC CCAGCGTCTT GTCATTGGCG 6780  
AATTCGAACA CGCAGATGCA GTCGGGGCGG CGCGGTCCCA GTCCCACTTC GCATATTAAG 6840  
GTGACGCGTG TGGCCTCGAA CACCGAGCGA CCCTGCAGCC AATATGGGAT CGGCCATTGA 6900  
ACAAGATGGA TTGCACGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT TCGGCTATGA 6960  
CTGGGCACAA CAGACAATCG GCTGCTCTGA TGCCGCCGTG TTCCGGCTGT CAGCGCAGGG 7020  
GCGCCCGGTT CTTTTGTCA AGACCGACCT GTCCGGTGCC CTGAATGAAC TGCAGGTAAG 7080  
TGCGGCCGTC GATGGCCGAG GCGGCCCTCGG CCTCTGCATA AATAAAAAA ATTAGTCAGC 7140  
CATGCATGGG GCGGAGAATG GCGGAACTG GCGGGAGTTA GGGCGGGAT GGGCGGAGTT 7200  
AGGGCGGGA CTATGGTTGC TGAATAATTG AGATGCATGC TTIGCATACT TCTGCCTGCT 7260  
GGGAGCCTG GGGACTTTCC ACACCTGGTT GCTGACTAAT TGAGATGCAT GCTTTGCATA 7320  
CTTCTGCCTG CTGGGAGCC TGGGGACTTT CCACACCCTA ACTGACACAC ATTCCACAGA 7380  
ATTAATTCCC CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA TAGCCCATAT 7440  
ATGGAGTTCC GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC 7500  
CCCCGCCCAT TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT AGGACTTTC 7560

# FIG. 8J

CATTGACGTC AATGGGTGGA CTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG 7620  
TATCATATGC CAAGTACGCC CCCTATTGAC GTCAATGACG GTAATGGCC CGCCTGGCAT 7680  
TATGCCCAGT ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA GCTATTAGTC 7740  
ATCGCTATTA CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG ATAGCGGTTT 7800  
GACTCACGGG GATTTCCAAG TCTCCACCCC ATTGACGTCA ATGGGAGTTT GTTTTGGCAC 7860  
CAAAATCAAC GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATTTGAC GCAAATGGGC 7920  
GGTAGGCGTG TACGGTGGGA GGTCTATATA AGCAGAGCTG GGTACGTGAA CCGTCAGATC 7980  
GCCTGGAGAC GCCATCACAG ATCTCTCACT ATGGATTTTC AGGTGCAGAT TATCAGCTTC 8040  
CTGCTAATCA GTGCTTCAGT CATAATGTCC AGAGGACAAA TTGTTCTCTC CCAGTCTCCA 8100  
GCAATCCTGT CTGCATCTCC AGGGGAGAAG GTCACAATGA CTTCAGAGGC CAGCTCAAGT 8160  
GTAAGTTACA TCCACTGGTT CCAGCAGAAG CCAGGATCCT CCCCCAAACC CTGGATTAT 8220  
GCCACATCCA ACCTGGCTTC TGGAGTCCCT GTTCGCTTCA GTGGCAGTGG GTCTGGGACT 8280  
TCTTACTCTC TCACAATCAG CAGAGTGGAG GCTGAAGATG CTGCCACTTA TTA CTGCCAG 8340  
CAGTGGAATA GTAACCCACC CACGTTCCGA GGGGGACCA AGCTGGAAAT CAAACGTACG 8400

# FIG. 8K

GTGGCTGCAC CATCTGTCTT CATCTTCCCG CCATCTGATG AGCAGTTGAA ATCTGGAAC 8460  
GCCTCTGTTG TGTGCCCTGCT GAATAACTTC TATCCAGAG AGGCCAAAGT ACAGTGGAAG 8520  
GTGGATAACG CCTCCAATC GGGTAACTCC CAGGAGAGTG TCACAGAGCA GGACAGCAAG 8580  
GACAGCACCT ACAGCCTCAG CAGCACCCCTG ACGCTGAGCA AAGCAGACTA CGAGAAACAC 8640  
AAAGTCTACG CCTGCGAAGT CACCCATCAG GGCCTGAGCT CGCCCGTCAC AAAGAGCTTC 8700  
AACAGGGGAG AGTGTTGAAT TCAGATCCGT TAACGGTTAC CAACTACCTA GACTGGATT 8760  
GTGACAACAT GCGGCCGTGA TATCTACGTA TGATCAGCCT CGACTGTGCC TTCTAGTTGC 8820  
CAGCCATCTG TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC 8880  
ACTGTCCCTT CCTAATAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCAATT 8940  
ATTCTGGGG GTGGGGTGGG GCAGGACAGC AAGGGGAGG ATTGGAAGA CAATAGCAGG 9000  
CATGCTGGGG ATGCGGTGGG CTCTATGGAA CCAGCTGGGG CTCGACAGCT ATGCCAAGTA 9060  
CGCCCCCTAT TGACGTCAAT GACGGTAAAT GGCCCGCCTG GCATTATGCC CAGTACATGA 9120  
CCTTATGGGA CTTTCCTACT TGGCAGTACA TCTACGTATT AGTCATCGCT ATTACCATGG 9180  
TGATGCGGTT TTGGCAGTAC ATCAATGGGC GTGGATAGCG GTTIGACTCA CGGGGATTTC 9240

# FIG. 8L

CAAGTCTCCA CCCCATTTGAC GTCAATGGGA GTTGTGTTTG GCACCAAAAT CAACGGGACT 9300  
TTCCAAAATG TCGTAACAAC TCCGCCCCAT TGACGCAAAT GGGCGGTAGG CGTGTACGGT 9360  
GGGAGGTCTA TATAAGCAGA GCTGGGTACG TCCTCACATT CAGTGATCAG CACTGAACAC 9420  
AGACCCGTCG ACATGGGTTG GAGCCTCATC TTGCTCTTCC TTGTCGCTGT TGCTACGCGT 9480  
GTCTGTCCC AGGTACAACT GCAGCAGCCT GGGGCTGAGC TGGTGAAGCC TGGGGCCTCA 9540  
GTGAAGATGT CCTGCAAGGC TTCTGGGTAC ACATTTACCA GTTACAATAT GCACTGGGTA 9600  
AAACAGACAC CTGGTCGGGG CCTGGAATGG ATTGGAGCTA TTTATCCCGG AAATGGTGAT 9660  
ACTTCCTACA ATCAGAAATT CAAAGGCAAG GCCACATTGA CTGCAGACAA ATCCTCCAGC 9720  
ACAGCCTACA TGCAGCTCAG CAGCCTGACA TCTGAGGACT CTGCGGTCTA TTAGTGTGCA 9780  
AGATCGACTT ACTACGGCGG TGA CTGGTAC TTCAATGTCT GGGGCGCAGG GACCACGGTC 9840  
ACCGTCTCTG CAGCTAGCAC CAAGGGCCCA TCGGTCTTCC CCCTGGCACC CTCCTCCAAG 9900  
AGCACCTCTG GGGGCACAGC GGCCCTGGGC TGCCTGGTCA AGGACTACTT CCCC GAACCG 9960  
GTGACGGTGT CGTGGAATC AGGCGCCCTG ACCAGCGGCG TGCACACCTT CCCGGCTGTC 10020  
CTACAGTCCT CAGGACTCTA CTCCTCAGC AGCGTGGTGA CCGTGCCCTC CAGCAGCTTG 10080

# FIG. 8M

GGCACCAGA CCTACATCTG CAACGTGAAT CACAAGCCCA GCAACACCAA GGTGGACAAG 10140  
AAAGCAGAGC CCAAATCTTG TGACAAAACT CACACATGCC CACCGTGCCC AGCACCTGAA 10200  
CTCCTGGGGG GACCGTCAGT CTTCCTCTTC CCCCCAAAC CCAAGGACAC CCTCATGATC 10260  
TCCCGGACCC CTGAGGTCAC ATGCGTGGTG GTGGACGTGA GCCACGAAGA CCCTGAGGTC 10320  
AAGTTCAACT GGTACGTGGA CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG 10380  
GAGCAGTACA ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG 10440  
CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC CCCCATCGAG 10500  
AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCCA 10560  
TCCCGGGATG AGCTGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTAT 10620  
CCCAGCGACA TCGCCGTGGA GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC 10680  
ACGCCTCCCG TGCTGGACTC CGACGGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGAC 10740  
AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC 10800  
AACCACTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA AATGAGGATC CGTTAACGGT 10860  
TACCAACTAC CTAGACTGGA TTCGTGACAA CATGCGGGCCG TGATATCTAC GTATGATCAG 10920

# FIG. 8N

CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCCTTCCT 10980  
TGACCCCTGGA AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA ATTGCATCGC 11040  
ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGTGGGGT GGGCAGGAC AGCAAGGGG 11100  
AGGATTGGGA AGACAATAGC AGGCATGCTG GGGATGCCGT GGGCTCTATG GAACCAGCTG 11160  
GGGCTCGACA GCAACGCTAG GTCGAGGCCG CTAATACTC TCTCTCCCT CCTTTTCCT 11220  
GCAGGACGAG GCAGCGCGG TATCGTGGCT GGCCACGACG GCGTTCCTT GCGCAGCTGT 11280  
GCTCGACGTT GTCACCTGAAG CGGGAAGGGA CTGGCTGCTA TTGGCGAAG TGCCGGGGCA 11340  
GGATCTCCTG TCATCTCACC TTGCTCCTGC CGAGAAAGTA TCCATCATGG CTGATGCAAT 11400  
GGGGCGGCTG CACACGCTTG ATCCGGGTAC CTGCCCATTC GACCACCAAG CGAAACATCG 11460  
CATCGAGCGA GCACGTACTC GGATGGAAGC CGGCTTTGTC GATCAGGATG ATCTGGACGA 11520  
AGAGCATCAG GGGCTCGGC CAGCCGAACT GTTCGCCAGG TAAGTGAGCT CCAATTCAAG 11580  
CTTCCTAGGG CGGCCAGCTA GTAGCTTTGC TTCTCAATT CTTATTGCA TAATGAGAAA 11640  
AAAAGGAAA TTAATTTTAA CACCAATTCA GTAGTTGATT GAGCAAATGC GTTGCCAAA 11700  
AGGATGCTTT AGAGACAGTG TTCTCTGCAC AGATAAGGAC AAACATTATT CAGAGGGAGT 11760

46/75

ACCCAGAGCT GAGACTCCTA AGCCAGTGAG TGGCACAGCA TTCTAGGGAG AAATATGCTT	11820
GTCATCACCG AAGCCTGATT CCGTAGAGCC ACACCTTGGT AAGGGCCAAT CTGCTCACAC	11880
AGGATAGAGA GGGCAGGAGC CAGGGCAGAG CATATAAGGT GAGGTAGGAT CAGTTGCTCC	11940
TCACATTTGC TTCTGACATA GTTGTTGG GAGCTTGGAT AGCTTGGACA GCTCAGGGCT	12000
GCGATTTTCGC GCCAAACTTG ACGGCAATCC TAGCGTGAAG GCTGGTAGGA TTTTATCCCC	12060
GCTGCCATCA TGGTTCGACC ATTGAACTGC ATCGTCGCCG TGTCCCAAAA TATGGGGATT	12120
GGCAAGAACG GAGACCTACC CTGGCCTCCG CTCAGGAACG AGTTCAAGTA CTTCCAAGA	12180
ATGACCACAA CCTCTTCAGT GGAAGGTAA CAGAACTCTGG TGATTATGGG TAGGAAAACC	12240
TGGTTCTCCA TTCCTGAGAA GAATCGACCT TTAAAGGACA GAATTAATAT AGTTCTCAGT	12300
AGAGAACTCA AAGAACCACC ACGAGGAGCT CATTTTCTTG CCAAAAGTTT GGATGATGCC	12360
TTAAGACTTA TTGAACAACC GGAATTGGCA AGTAAAGTAG ACATGGTTTG GATAGTCGGA	12420
GGCAGTTCTG TTTACCAGGA AGCCATGAAT CAACCAGGCC ACCTTAGACT CTTTGTGACA	12480
AGGATCATGC AGGAATTTGA AAGTGACACG TTTTCCCCAG AAATTGATTT GGGGAAATAT	12540
AAACTTCTCC CAGAATACCC AGGCGTCCTC TCTGAGGTCC AGGAGGAAA AGGCATCAAG	12600

# FIG. 8Q

TATAAGTTTG AAGTCTACGA GAAGAAAGAC TAACAGGAAG ATGCTTTCAA GTTCTCTGCT 12660  
CCCCTCCTAA AGCTATGCAT TTTTATAAGA CCATGGGACT TTTGCTGGCT TTAGATCAGC 12720  
CTCGACTGTG CTTTCTAGTT GCCAGCCATC TGTTGTTTGC CCCTCCCCCG TGCCCTTCCTT 12780  
GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA AATGAGGAAA TTGCATCGCA 12840  
TTGTCIGAGT AGGTGTCAAT CTATTCTGGG GGGTGGGGTG GGGCAGGACA GCAAGGGGGA 12900  
GGATTGGAA GACAATAGCA GGATGCTGG GGATGCGGTG GGCTCTATGG AACCAGCTGG 12960  
GGCTCGAAGC GGCCGCCCAT TTCGCTGGTG GTCAGATGCG GGATGGCGTG GGACGCGGCG 13020  
GGGAGCGTCA CACTGAGGTT TTCCGCCAGA CGCCACTGCT GCCAGGCGCT GATGTGCCCG 13080  
GCTTCTGACC ATGCGGTCGC GTTCGGTTGC ACTACGCGTA CTGTGAGCCA GAGTTGCCCG 13140  
GGCTCTCCG GCTGCGGTAG TTCAGGCAGT TCAATCAACT GTTTACCTTG TGAACCGACA 13200  
TCCAGAGGCA CTTACCCGCT TGCCAGCGGC TTACCATCCA GCGCCACCAT CCAGTGCAGG 13260  
AGCTCGTTAT CGCTATGACG GAACAGGTAT TCGCTGGTCA CTTCGATGGT TTGCCCGGAT 13320  
AAACGGAAC TGAAGAACTG CTGCTGGTGT TTTGCTTCCG TCAGCGCTGG ATGCGGCGTG 13380  
CGGTCGGCAA AGACCAGACC GTTCATACAG AACTGGCGAT CGTTCCGGCT ATGCCCAAAA 13440

# FIG. 8R

TCACCGCCGT AAGCCGACCA CGGGTTGCCG TTTTCATCAT ATTTAATCAG CGACTGATCC 13500  
ACCCAGTCCC AGACGAAGCC GCCCTGTAA CGGGATACT GACGAAACGC CTGCCAGTAT 13560  
TTAGCGAAAC CGCCAAGACT GTTACCCATC GCTGGGGCGT ATTCGCAAAG GATCAGCGGG 13620  
CGCGTCTCTC CGGGTAGCGA AAGCCATTTT TTGATGGACC ATTTCCGGACC AGCCGGGAAG 13680  
GGCTGGTCTT CATCCACGCG CGCGTACATC GGGCAAATAA TATCGGTGGC CGTGGTGTCTG 13740  
GCTCCGCCGC CTTCATACTG CACCGGGCGG GAAGGATCGA CAGATTTGAT CCAGCGATAC 13800  
AGCGGTCGT GATTAGCGC GTGGCCTGAT TCATTCCCA GCGACCAGAT GATCACACTC 13860  
GGGTGATTAC GATCGCGCTG CACCATTCGC GTTACGCGT CGCTCATCGC CGGTAGCCAG 13920  
CGCGGATCAT CGGTCAGACG ATTCATTGGC ACCATGCCGT GGGTTTCAAT ATTGGCTTCA 13980  
TCCACCACAT ACAGGCCGTA GCGGTCGCAC AGCGTGTACC ACAGCGGATG GTTCGGATAA 14040  
TGCCAACAGC GCACGGCGTT AAAGTTGTTT TGCTTCATCA GCAGGATATC CTGCACCATC 14100  
GTCTGCTCAT CCATGACCTG ACCATGCAGA GGATGATGCT CGTGACGGTT AACGCCCTCGA 14160  
ATCAGCAACG GCTTGCCGTT CAGCAGCAGC AGACCATTTT CAATCCGCAC CTCGCGGAAA 14220  
CCGACATCGC AGGCTTCTGC TTCAATCAGC GTGCCGTCGG CGGTGTGCAG TTCAACCACC 14280

# FIG. 8S

GCACGATAGA GATTGGGGAT TTCGGCGCTC CACAGTTTCG GGTTTTCGAC GTTCAGACGC 14340  
AGTGTGACGC GATCGGCATA ACCACCACGC TCATCGATAA TTTCACCGCC GAAAGCGCGG 14400  
GTGCCGCTGG CGACCTGCGT TTCACCCCTGC CATAAAGAAA CTGTTACCCG TAGGTAGTCA 14460  
CGCAACTCGC CGCACATCTG AACTTCAGCC TCCAGTACAG CGCGGCTGAA ATCATCATTA 14520  
AAGCGAGTGG CAACATGGAA ATCGCTGATT TGTGTAGTCG GTTTATGCAG CAACGAGACG 14580  
TCACGGAAA TGCCGCTCAT CCGCCACATA TCCTGATCTT CCAGATAACT GCCGTCACTC 14640  
CAACGCAGCA CCATCACCGC GAGGCGGTTT TCTCCGGCGC GTAAAAATGC GCTCAGGTCA 14700  
AATTCAGACG GCAAACGACT GTCCTGGCCG TAACCGACCC ACGCCCCGTT GCACCACAGA 14760  
TGAAACGCCG AGTTAACGCC ATCAAAAATA ATTGCGTCT GGCCTTCCTG TAGCCAGCTT 14820  
TCATCAACAT TAAATGTGAG CGAGTAACAA CCCGTCGGAT TCTCCGTGGG AACAAACGGC 14880  
GGATTGACCG TAATGGGATA GGTACGTTG GTGTAGATGG GCGCATCGTA ACCGTGCATC 14940  
TGCCAGTTTG AGGGGACGAC GACAGTATCG GCCTCAGGAA GATCGCACTC CAGCCAGCTT 15000  
TCCGGCACCG CTTCTGGTGC CGGAAACCAG GCAAAGCGCC ATTGCCATT CAGGCTGCCG 15060  
AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT TACGCCAGCT GCGAAAGGG 15120

# FIG. 8T

GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT TTCCACAGTC ACGACGTTGT 15180  
AAACGACTT AATCCGTCGA GGGGCTGCCT CGAAGCAGAC GACCTTCCGT TGTGCAGCCA 15240  
GCGGCGCCTG CGCCGGTGCC CACAATCGTG CGCGAACAAA CTAAACCAGA ACAAATTATA 15300  
CCGCGGCAC CGCCGCCACC ACCTTCTCCC GTGCCATAACA TTCCAGCGCC TCCACCACCA 15360  
CCACCACCAT CGATGTCTGA ATTGCCGCC GCTCCACCAA TGCCGACGGA ACCTCAACCC 15420  
GCTGCACCTT TAGACGACAG ACAACAATTG TTGGAAGCTA TTAGAAACGA AAAAAATCGC 15480  
ACTCGTCTCA GACCGGTCAA ACCAAAAACG GCGCCCGAAA CCAGTACAAT AGTTGAGGTG 15540  
CCGACTGTGT TGCCTAAAGA GACATTTGAG CCTAAACCGC CGTCTGCATC ACCGCCACCA 15600  
CCTCCGCCCTC CGCCTCCGCC GCCAGCCCCG CCTGGCCCTC CACCGATGGT AGATTTATCA 15660  
TCAGCTCCAC CACCGCCGCC ATTAGTAGAT TTGCCGTCTG AAATGTTACC ACCGCCTGCA 15720  
CCATCGCTTT CTAACGTGTT GTCTGAATTA AAATCGGGCA CAGTTAGATT GAAACCCGCC 15780  
CAAAAACGCC CGCAATCAGA AATAATTCCA AAAAGCTCAA CTACAAATTT GATCGCGGAC 15840  
GTGTTAGCCG ACACAATTAA TAGGCGTCGT GTGGCTATGG CAAATCGTC TTCGGAAGCA 15900  
ACTTCTAACG ACGAGGGTTG GGACGACGAC GATAATCGGC CTAATAAAGC TAACACGCC 15960

50/75

# FIG. 8U

GATGTTAAAT ATGTCCAAGC TACTAGTGGT ACCGCTTGGC AGAACATATC CATCGCGTCC 16020  
GCCATCTCCA GCAGCCGCAC GCGGCGCATC TCGGCGAGCG TTGGGTCTCTG GCCACGGGTG 16080  
CGCATGATCG TGCTCCTGTC GTTGAGGACC CCGCTAGGCT GGCGGGGTTG CCTTACTGGT 16140  
TAGCAGAATG AATCACCGAT ACGCGAGCGA ACGTGAAGCG ACTGCTGCTG CAAAACGTCT 16200  
GCGACCTGAG CAACAACATG AATGGTCTTC GGTTCCTG TTTGTAAG TCTGAAACG 16260  
CGGAAGTCAG CGCCCTGCAC CATTATGTTT CCGATCTGCA TCGCAGGATG CTGCTGGCTA 16320  
CCCTGTGGAA CACCTACATC TGTATTAACG AAGCGCTGGC ATTGACCCCTG AGTGATTTTT 16380  
CTCTGGTCCC GCCGCATCCA TACCGCCAGT TGTTTACCCT CACAACGTTT CAGTAACCCG 16440  
GCATGTTTAT CATCAGTAAC CCGTATCGTG AGCATCCTCT CTCGTTTCAT CGGTATCAT 16500  
ACCCCATGA ACAGAAATCC CCTTACACG GAGGCATCAG TGACCAAACA GGAAAAAAC 16560  
GCCCTTAACA TGGCCCGCTT TATCAGAAGC CAGACATTAA CGCTTCTGGA GAACTCAAC 16620  
GAGCTGGACG CGGATGAACA GGCAGACATC TGTGAATCGC TTCACGACCA CGCTGATGAG 16680  
CTTTACCGCA GCTGCCTCGC GCGTTTCGGT GATGACGGTG AAAACCTCTG ACACATGCAG 16740  
CTCCCCGAGA CCGTCACAGC TTGCTGTAA GCGGATGCCG GGAGCAGACA AGCCCCGTCAG 16800

# FIG. 8V

GGCGGTCAG CGGGTGTTGG CGGGTGTCGG GCGCAGCCA TGACCCAGTC ACGTAGCGAT 16860  
AGCGGAGTGT AACTATGCGG CATCAGAGCA GATTGACTG AGAGTGCACC 16920  
ATATGCGGTG TGAATAACCG CACAGATGCG TAAGGAGAAA ATACCGCATC AGGCGCTCTT 16980  
CCGCTTCCTC GCTCACTGAC TCGCTGCGCT CCGTCGTTCC GCTGCGGCGA GCGGTATCAG 17040  
CTCACTCAA GCGGGTAATA CGGTTATCCA CAGATCAGG GGATAACGCA GGAAGAACA 17100  
TGTGAGCAA AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT 17160  
TCCATAGGCT CCGCCCCCCT GACGAGCATC ACAAATCG ACGCTCAAGT CAGAGGTGGC 17220  
GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAAGCTCC CTCGTGCGCT 17280  
CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT TCGGGAAGCG 17340  
TGGCGCTTTC TCATAGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC GTTCGCTCCA 17400  
AGCTGGGCTG TGTGCACGAA CCCCCCGTTC AGCCCGACCG CTGCGCCTTA TCCGGTAACT 17460  
ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA GCCACTGGTA 17520  
ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG TGGTGGCCTA 17580  
ACTACGGCTA CACTAGAAGG ACAGTATTG GTATCTGCGC TCTGCTGAAG CCAGTTACCT 17640

# FIG. 8W

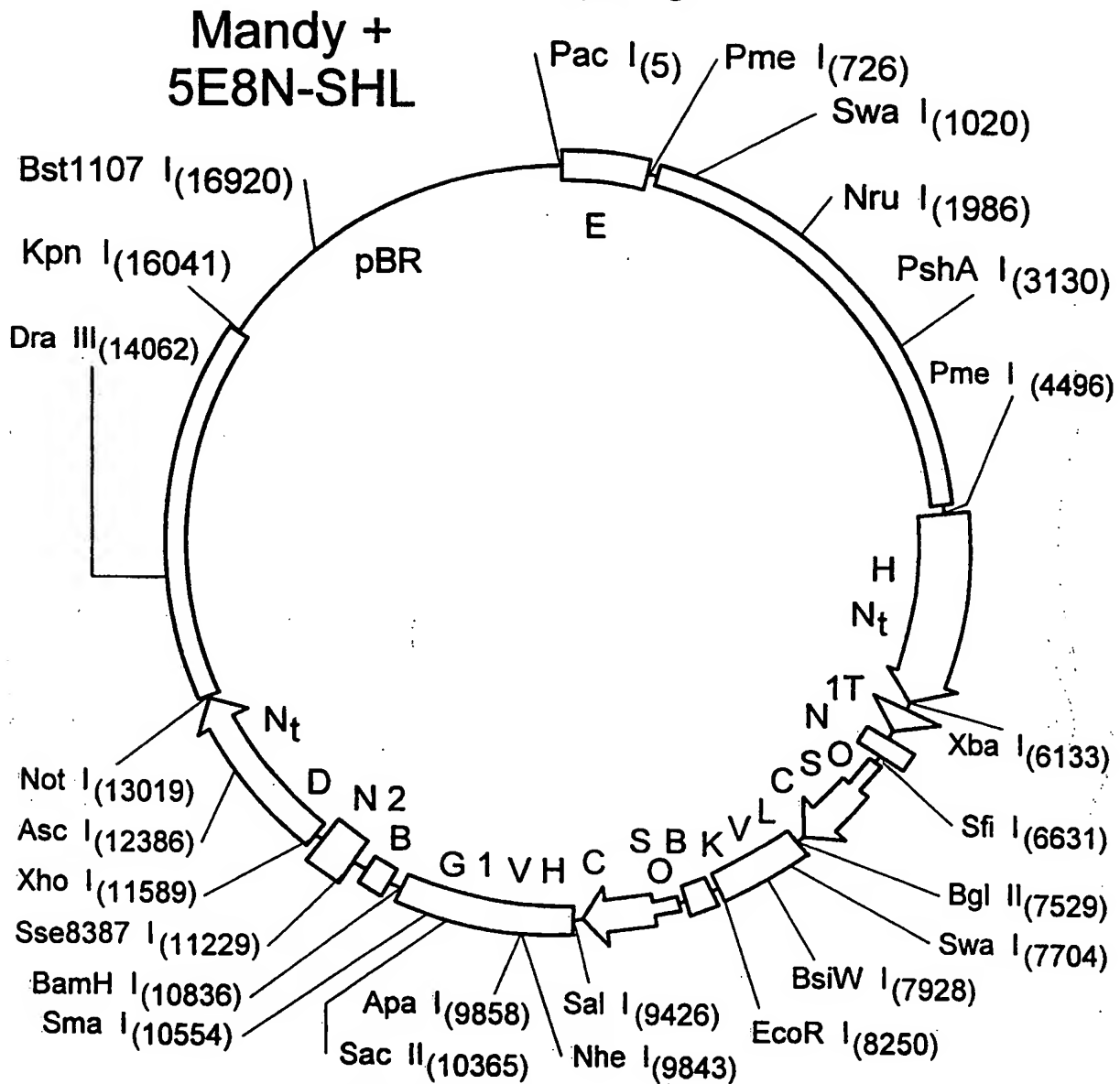
TCGGAAAAG AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGTGGTT 17700  
TTTTTGTTTG CAAGCAGCAG ATTACGCGCA GAAAAAAGG ATCTCAAGAA GATCCTTTGA 17760  
TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAACTC ACGTTAAGGG ATTTTGGTCA 17820  
TGAGATTATC AAAAAGGATC TTCACCTAGA TCCTTTTAAA TTAAAAATGA AGTTTTAAAT 17880  
CAATCTAAAG TATATATGAG TAAACTTGGT CTGACAGTTA CCAATGCTTA ATCAGTGAGG 17940  
CACCTATCTC AGCGATCTGT CTATTTTCGT CATCCATAGT TGCCTGACTC CCCGTCGTGT 18000  
AGATACTAC GATACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG ATACCGCGAG 18060  
ACCCACGCTC ACCGGCTCCA GATTATCAG CAATAAACCA GCCAGCCGGA AGGGCCGAGC 18120  
GCAGAAAGTG TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT TGCCGGGAAG 18180  
CTAGAGTAAG TAGTTCGCCA GTTAATAGTT TGCGCAACGT TGTTGCCATT GCTGCAGGCA 18240  
TCGTGGTGTC ACGCTCGTCG TTTGGTATGG CTTCAATTCAG CTCGGGTTCC CAACGATCAA 18300  
GGCGAGTTAC ATGATCCCCC ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC GGTCCTCCGA 18360  
TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT TATCACTCAT GGTATGGCA GCACTGCATA 18420  
ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG TACTCAACCA 18480

# FIG. 8X

AGTCATTCTG AGAATAGTGT ATGCGGCGAC CGAGTTGCTC TTGCCCGGCG TCAACACGGG 18540  
ATAATACCGC GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA CGTTCTTCGG 18600  
GGCGAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA CCCACTCGTG 18660  
CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG 18720  
GAAGGCAAA TGCCGC AAAA AAGGGAATAA GGGCGACACG GAAATGTTGA ATACTCATAC 18780  
TCTTCCTTT TCAATATTAT TGAAGCATTT ATCAGGGTTA TTGTCTCATG AGCGGATACA 18840  
TATTGAATG TATTAGAAA AATAAACAAA TAGGGTTCC GCGCACATTT CCCC GAAAAG 18900  
TGCCACCTGA CGTCTAAGAA ACCATTATTA TCATGACATT AACCTATAAA AATAGGCGTA 18960  
TCACGAGGCC CTTTCGTCTT CAAGAA 18986

54/75

55/75

**FIG. 9**

Nt D = Inactive Dihydrofolate reductase

E = CMV and SV40 enhancers

Nt H = Inactive Salmonella Histidinol Dehydrogenase

T = Herpes Simplex thymidine kinase promoter and polyoma enhancer

C = Cytomegalovirus promoter/enhancer

N1 = Neomycin phosphotransferase exon 1

K = Human kappa constant

VL = Variable light chain anti-CD23 primate 5E8 and leader

VH = Variable heavy chain anti-CD-23 primate 5E8N- and leader

B = Bovine growth hormone polyadenylation

M2 = Neomycin phosphotransferase exon 2

G1 = Human Gamma 1 constant

Mandy cut Xba I and ligated to Xba I Xho I fragment

from XKG1+CD23 5E8N-SHL

Map by Mitchell Reff Constructed by Karen McLachlan 06/26/97 19,035 bp

Noncutters = AflII, AvrII, HindIII, I-PpoI, I-SceI, PmlI, RsrII, SgfI, SrfI

56/75

10	20	30	40	50	60	70
TTAATTAAGG	GGCGGAGAAT	GGCGGGAAT	GGCGGGAGTT	AGGGCGGGGA	TGGGCGGAGT	TAGGGGCGGG
80	90	100	110	120	130	140
ACTATGGTTG	CTGACTAATT	GAGATGCATG	CTTTGCATAC	TTCTGCCTGC	TGGGAGGCCT	GGGACTTTTC
150	160	170	180	190	200	210
CACACCTGGT	TGCTGACTAA	TTGAGATGCA	TGCTTTGCAT	ACTTCTGCCT	GCTGGGGAGC	CTGGGGACTT
220	230	240	250	260	270	280
TCCACACCCCT	AACTGACACA	CATTCCACAG	AATTAATTCC	CCTAGTTATT	AATAGTAATC	AATTACGGGG
290	300	310	320	330	340	350
TCATTAGTTC	ATAGCCCAT	TATGGAGTTC	CGCGTTACAT	AAC TTACGGT	AAATGGCCCG	CCTGGCTGAC
360	370	380	390	400	410	420
CGCCCAACGA	CCCCCGCCCA	TTGACGTCAA	TAATGACGTA	TGTTCCCAT	GTAACGCCAA	TAGGGACTTT
430	440	450	460	470	480	490
CCATTGACGT	CAATGGGTGG	AGTATTTACG	GTAAACTGCC	CACTTGGCAG	TACATCAAGT	GTATCATATG
500	510	520	530	540	550	560
CCAAGTACGC	CCCCTATTGA	CGTCAATGAC	GGTAAATGGC	CCGCCCTGGCA	TTATGCCCCAG	TACATGACCT
570	580	590	600	610	620	630
TATGGGACTT	TCCTACTTGG	CAGTACATCT	ACGTATTAGT	CATCGCTATT	ACCATGGTGA	TGCGGTTTTG
640	650	660	670	680	690	700
GCAGTACATC	AATGGGCGTG	GATAGCGGTT	TGACTCACGG	GGATTTCCAA	GTCTCCACCC	CATTGACGTC
710	720	730	740	750	760	770
AATGGGAGTT	TGTTTTGAAG	CTGTTTAAAC	AGCTTGGCCG	GCCAGCTTTA	TTTAAACGTGT	TTACGTCGAG
780	790	800	810	820	830	840
TCAATTGTAC	ACTAACGACA	GTGATGAAAG	AAATACAAAA	GCGCATAATA	TTTTGAACGA	CGTCGAACCT
850	860	870	880	890	900	910
TTATTACAAA	ACAAAACACA	AACGAATATC	GACAAAGCTA	GATTGCTGCT	ACAAGATTTG	GCAAGTTTTG
920	930	940	950	960	970	980
TGGCGTTGAG	CGAAAATCCA	TTAGATAGTC	CAGCCATCGG	TTCGGAAAAA	CAACCCTTGT	TTGAAACTAA

FIG. 10A

57/75

TCGAAACCTA	990	TTTTACAAAT	1000	CTATTGAGGA	1010	TTTAATATTT	1020	AAATTCAGAT	1030	ATAAAGACGC	1040	TGAAAATCAT	1050
TTGATTTTCG	1060	CTCTAACATA	1070	CCACCCCTAAA	1080	GATTATAAAT	1090	TTAATGAAT	1100	ATTAAAATAC	1110	ATCAGCAACT	1120
ATATATTGAT	1130	AGACATTTCC	1140	AGTTTGAT	1150	ATTAGTTTGT	1160	CGGTCTCAT	1170	ACAATGGCTG	1180	TTATTTTIAA	1190
CAACAAACAA	1200	CTGCTCGCAG	1210	ACAATAGTAT	1220	AGAAAAGGGA	1230	GGTGAACGT	1240	TTTTGTTTAA	1250	CGGTTCGTAC	1260
AACATTTTGG	1270	AAAGTTATGT	1280	TAATCCGGTG	1290	CTGCTAAAAA	1300	ATGGTGTAAT	1310	TGAAC TAGAA	1320	GAAGCTGCGT	1330
ACTATGCCGG	1340	CAACATATTG	1350	TACAAAACCG	1360	ACGATCCCAA	1370	ATTCATTGAT	1380	TATATAAAT	1390	TAATAATTAA	1400
AGCAACACAC	1410	TCCGAAGAAC	1420	TACCAGAAAA	1430	TAGCACTGTT	1440	GTAAATTACA	1450	GAAAAAATAT	1460	GCGCAGCGGT	1470
ACTATACACC	1480	CCATTAAAAA	1490	AGACATATAT	1500	ATTTATGACA	1510	ACAAAAAAT	1520	TACTCTATAC	1530	GATAGATACA	1540
TATATGGATA	1550	CGATAATAAC	1560	TATGTTAATT	1570	TTTATGAGGA	1580	GAAAAATGAA	1590	AAAGAGAAGG	1600	AATACGAAGA	1610
AGAAGACGAC	1620	AAGGCGTCTA	1630	GTTTATGTGA	1640	AAATAAAAT	1650	ATATTGTCGC	1660	AAATTAACTG	1670	TGAATCATTT	1680
GAAATGATT	1690	TTAAATATTA	1700	CCTCAGCGAT	1710	TATAACTACG	1720	CGTTTTCAT	1730	TATAGATAAT	1740	ACTACAAAATG	1750
TTCTTGTTGC	1760	GTTTGTTTG	1770	TATCGTTAAT	1780	AAAAAACAAA	1790	TTTGACATTT	1800	ATAATTGTTT	1810	TATTATTCAA	1820
TAATTACAAA	1830	TAGGATTGAG	1840	ACCCTTGCAG	1850	TTGCCAGCAA	1860	ACGGACAGAG	1870	CTTGTCGAGG	1880	AGAGTTGTTG	1890
ATTCATTGTT	1900	TGCCTCCCTG	1910	CTGCGGTTTT	1920	TCACCGAAGT	1930	TCATGCCAGT	1940	CCAGCGTTTT	1950	TGCAGCAGAA	1960

FIG. 10B

58/75

1970	1980	1990	2000	2010	2020	2030
AAGCCGCCGA	CTTCGGTTTG	CGGTCGGCGAG	TGAAGATCCC	TTTCTTGTTA	CCGCCAACGC	GCAATATGCC
2040	2050	2060	2070	2080	2090	2100
TTGCGAGGTC	GCAAAATCGG	CGAAATTCCA	TACCTGTTCA	CCGACGACGG	CGCTGACGCG	ATCAAAGACG
2110	2120	2130	2140	2150	2160	2170
CGGTGATACA	TATCCAGCCA	TGCACACTGA	TACTCTTCAC	TCCACATGTC	GGTGATACAT	GAGTGCAGCC
2180	2190	2200	2210	2220	2230	2240
CGGCTAACGT	ATCCACGCCG	TATTCGGTGA	TGATAATCGG	CTGATGCAGT	TTCTCCTGCC	AGGCCAGAAC
2250	2260	2270	2280	2290	2300	2310
TTCTTTTTC	AGTACCTTCT	CTGCCGTTTC	CAAATCGCCG	CTTTGGACAT	ACCATCCGTA	ATAACGGTTC
2320	2330	2340	2350	2360	2370	2380
AGGCACAGCA	CATCAAAGAG	ATCGCTGATG	GTATCGGTGT	GAGCGTCGCA	GAACATTACA	TTGACGCAGG
2390	2400	2410	2420	2430	2440	2450
TGATCGGACG	CGTCGGGTCC	AGTTTACGCG	TTGCTTCCGC	CAGTGGCGCG	AAATATTCCC	GTGCACCTTG
2460	2470	2480	2490	2500	2510	2520
CGGACGGGTA	TCCGGTTCGT	TGGCAATACT	CCACATCACG	ACGCTTGGGT	GGTTTTTGTC	ACGCGCTATC
2530	2540	2550	2560	2570	2580	2590
AGCTCTTTAA	TCGCCGTGTA	GTGCGCTTGC	TGAGTTTCCC	CGTTGACTGC	CTCTTCGCTG	TACAGTTCTT
2600	2610	2620	2630	2640	2650	2660
TCGGCTTGTT	GCCCGCTTCG	AAACCAATGC	CTAAAGAGAG	GTTAAAGCCG	ACAGCAGCAG	TTTCATCAAT
2670	2680	2690	2700	2710	2720	2730
CACCACGATG	CCATGTTTCA	CTGCCCAGTC	GAGCATCTCT	TCAGCGTAAG	GGTAATGCCA	GGTACGGTAG
2740	2750	2760	2770	2780	2790	2800
GAGTTGGCCC	CAATCCAGTC	CATTAATGCG	TGGTCGTGCA	CCATCAGCAC	GTTATCGAAT	CCTTTGCCAC
2810	2820	2830	2840	2850	2860	2870
GCAAGTCCGC	ATCTTCATGA	CGACCAAAGC	CAGTAAAGTA	GAACGGTTTG	TGGTTAATCA	GGAACGTGTC
2880	2890	2900	2910	2920	2930	2940
GCCCTTCACT	GCCACTGACC	GGATGCCGAC	GCGAAGCGGG	TAGATATCAC	ACTCTGTCTG	GCTTTTGGCT

FIG. 10C

59/75

2950	GTGACGCACA	2960	GTTCATAGAG	2970	ATAACCTTCA	2980	CCCGGTTGCC	2990	AGAGGTGCGG	3000	ATTCACCACT	3010	TGCAAAAGTCC
3020	CGCTAGTGCC	3030	TTGTCCAGTT	3040	GCAACCACTT	3050	GTTGATCCGC	3060	ATCACGCAGT	3070	TCAACGCTGA	3080	CATCACCACT
3090	GGCCACCACC	3100	TGCCAGTCAA	3110	CAGACGCGTG	3120	GTTACAGTCT	3130	TGCGCGGACAT	3140	GCGTCACCCAC	3150	GGTGATATCG
3160	TCCACCCAGG	3170	TGTTCGGCGT	3180	GGGTAGAGC	3190	ATTACGCTGC	3200	GATGGATTCC	3210	GGCATAGTTA	3220	AAGAAATCAT
3230	GGAAGTAAGA	3240	CTGCTTTTTC	3250	TTGCCGTTTT	3260	CGTCGGTAAT	3270	CACCAATCCC	3280	GGCGGGATAG	3290	TCTGCCAGTT
3300	CAGTTCGTTG	3310	TTCACACAAA	3320	CGGTGATACC	3330	CCTCGACGGA	3340	TAAAGACTT	3350	CAAGCGGTCA	3360	ACTATGAAGA
3370	AGTGTTTCGTC	3380	TTCGTCCAG	3390	TAAGCTATGT	3400	CTCCAGAATG	3410	TAGCCATCCA	3420	TCCTTGTCAA	3430	TCAAGGCGGT
3440	GGTCGCTTCC	3450	GGATTGTTTA	3460	CATAACCGGA	3470	CATAATCATA	3480	GGTCCCTCTGA	3490	CACATAATTC	3500	GCCTCTCTGA
3510	TTAACGCCCA	3520	GCGTTTTCCC	3530	GGTATCCAGA	3540	TCCACAACCT	3550	TCGCTTCAAA	3560	AAATGGAACA	3570	ACTTTACCGA
3580	CCGCGCCCCGG	3590	TTTATCATCC	3600	CCCTCGGGTG	3610	TAATCAGAAAT	3620	AGCTGATGTA	3630	GTCTCAGTGA	3640	GCCCATATCC
3650	TTGTCGTATC	3660	CCTGGAAGAT	3670	GGAAGCGTTT	3680	TGCAACCGCT	3690	TCCCAGACTT	3700	CTTTCGAAAG	3710	AGGTGCGCCCC
3720	CCAGAAGCAA	3730	TTTCGTGTAA	3740	ATTAGATAAA	3750	TCGTATTTGT	3760	CAATCAGAGT	3770	GCITTTGGCG	3780	AAGAATGAAA
3790	ATAGGGTTGG	3800	TACTAGCAAC	3810	GCACTTTTGA	3820	TTTTGTAATC	3830	CTGAAGGGAT	3840	CGTAAAAACA	3850	GCTCTTCTTC
3860	AAATCTATAC	3870	ATTAAGACGA	3880	CTCGAAATCC	3890	ACATATCAAA	3900	TATCCGAGTG	3910	TAGTAAACAT	3920	TCCAAAAACCG

FIG. 10D

60/75

3930	3940	3950	3960	3970	3980	3990
TGATGGAATG	GAACAACACT	TAAAATCGCA	GTATCCGGAA	TGATTTGATT	GCCAAAATA	GGATCTCTGG
4000	4010	4020	4030	4040	4050	4060
CATGCGAGAA	TCTGACGCAG	GCAGTTCTAT	GCGGAAGGGC	CACACCCCTTA	GGTAACCCAG	TAGATCCAGA
4070	4080	4090	4100	4110	4120	4130
GGAAATTGTT	TGTCACGATC	AAAGGACTCT	GGTACAAAAT	CGTATTCAAT	AAAACCGGGA	GGTAGATGAG
4140	4150	4160	4170	4180	4190	4200
ATGTGACGAA	CGTGTACATC	GACTGAAATC	CCTGGTAATC	CGTTTTAGAA	TCCATGATAA	TAATTTTCTG
4210	4220	4230	4240	4250	4260	4270
GATTATTGGT	AATTTTTTTT	GCACGTTCAA	AATTTTTTGC	AACCCCTTTT	TGGAAACAAA	CACTACGGTA
4280	4290	4300	4310	4320	4330	4340
GGCTGCGAAA	TGTTTCATACT	GTTGAGCAAT	TCACGTTTCA	TATAAATGTC	GTTTCGCGGC	GCAACTGCAA
4350	4360	4370	4380	4390	4400	4410
CTCCGATAAA	TAACGCGCCC	AACACCGGCA	TAAAGAAATG	AAGAGAGTTT	TCACTGCATA	CGACGATTCT
4420	4430	4440	4450	4460	4470	4480
GTGATTTGTA	TTCAGCCCAT	ATCGTTTCAT	AGCTTCTGCC	AACCGAACGG	ACATTTTCGAA	GTATTTCCGCG
4490	4500	4510	4520	4530	4540	4550
TACAGCCCCG	CCGTTTAAAC	GGCCGGGCTT	CAATACCCCTG	ATTGACTGGA	ACAGCTGTAG	CCCTGAACAG
4560	4570	4580	4590	4600	4610	4620
CAGCGTGCGC	TGCTGACGGG	TCCGGCGATT	TCCGCCCTCTG	ACAGTATTAC	CCGGACGGTC	AGCGATATTC
4630	4640	4650	4660	4670	4680	4690
TGGATAATGT	AAAAACGCGC	GGTGACGATG	CCCTGCGTGA	ATACAGCGCT	AAATTTGATA	AAACAGAAAT
4700	4710	4720	4730	4740	4750	4760
GACAGCGCTA	CGCGTCACCC	CTGAAGAGAT	CGCCGCCGCC	GGCGCGCGTC	TGAGCGACGA	ATTAAAACAG
4770	4780	4790	4800	4810	4820	4830
GCGATGACCG	CTGCCGTCAA	AAATATTGAA	ACGTTCCATT	CCGCGCAGAC	GCTACCGCCT	GTAGATGTGG
4840	4850	4860	4870	4880	4890	4900
AAACCCAGCC	AGGCGTGCCT	TGCCAGCAGG	TTACGCGTCC	CGTCTCGTCT	GTCGGTCTGT	ATATTCCCGG

FIG. 10E

61/75

CGGCTCGGCT	4910	CCGCTCTTCT	4920	CAACGGTGCT	4930	GATGCTGGCG	4940	ACGCCGGCGC	4950	GCATTGCGGG	4960	ATGCCAGAAAG	4970
GTGGTTCTGT	4980	GCTCGCCGCC	4990	GCCCATCGCT	5000	GATGAAATCC	5010	TCTATGCGGC	5020	GCAACTGTGT	5030	GGCGTGACGG	5040
AAATCTTTAA	5050	CGTCGGCCGC	5060	GCGCAGGCCA	5070	TTTGCCGCTCT	5080	GGCCTTCGGC	5090	AGCGAGTCCG	5100	TACCGAAAAGT	5110
GGATAAAATT	5120	TTTGCCCCCG	5130	GCAACGCCTT	5140	TGTAACCGAA	5150	GCCAAACGTC	5160	AGGTCAGCCA	5170	GCGTCTCGAC	5180
GGCGCGGCTA	5190	TCGATATGCC	5200	AGCCGGGCGG	5210	TCTGAAGTAC	5220	TGGTGATCGC	5230	AGACAGCGGC	5240	GCAACACCGG	5250
ATTCGCTGC	5260	TTCTGACCTG	5270	CTCTTCCCAGG	5280	CTGAGCACGG	5290	CCCGGATTCC	5300	CAGGTGATCC	5310	TGCTGACGCC	5320
TGATGCTGAC	5330	ATTGCCCGCA	5340	AGGTGGCGGA	5350	GGCGGTAGAA	5360	CGTCAACTGG	5370	CGGAACTGCC	5380	GCGCGCGGAC	5390
ACCGCCCGGC	5400	AGGCCCTGAG	5410	CGCCAGTCGT	5420	CTGATTGTGA	5430	CCAAAGATTT	5440	AGCGCAGTGC	5450	GTCGCCATCT	5460
CTAATCAGTA	5470	TGGGCCGGAA	5480	CACCTTAATCA	5490	TCCAGACGCG	5500	CAATGCGCGC	5510	GATTTGGTGG	5520	ATGCGATTAC	5530
CAGCGCAGGC	5540	TCGGTATTTT	5550	TCGGCGACTG	5560	GTCGCCGGAA	5570	TCCGCCGGTG	5580	ATTACGCTTC	5590	CGGAACCAAC	5600
CATGTTTTAC	5610	CGACCTATGG	5620	CTATACTGCT	5630	ACCTGTTCCA	5640	GCCTTGGGTT	5650	AGCGGATTTT	5660	CAGAAACGGA	5670
TGACCGTTCA	5680	GGAACGTGCG	5690	AAAGCGGGCT	5700	TTTCCGCTCT	5710	GGCATCAACC	5720	ATTGAAACAT	5730	TGGCGGCGGC	5740
AGAACGTCTG	5750	ACCGCCCAT	5760	AAAATGCCGT	5770	GACCCCTGCGC	5780	GTAAACGCCC	5790	TCAAGGAGCA	5800	AGCATGAGCA	5810
CTGAAAACAC	5820	TCTCAGCGTC	5830	GCTGACTTAG	5840	CCCCTGAAAA	5850	TGTCCGCAAC	5860	CTGGAGATCC	5870	AGACATGGAT	5880

FIG. 10F

62/75

5890	AAGATACATT	5900	GATGAGTTTG	5910	GACAAACCAC	5920	AACTAGAATG	5930	CAGTGAAAAA	5940	AATGCTTTAT	5950	TTGTGAAATT
5960	TGTGATGCTA	5970	TTGCTTTATT	5980	TGTAACCATT	5990	ATAAGCTGCA	6000	ATAACAAGT	6010	TAACAACAAC	6020	AATTGCATTG
6030	ATTTTATGTT	6040	TCAGGTTTCAG	6050	GGGGAGGTGT	6060	GGGAGGTTTT	6070	TTAAAGCAAG	6080	TAAACCTCT	6090	ACAAATGTGG
6100	TATGGCTGAT	6110	TATGATCTCT	6120	AGGGCCGGCC	6130	CTCGACGGCG	6140	CGTCTAGAGC	6150	AGTGTGGTTT	6160	TCAAGAGGAA
6170	GCAAAAAGCC	6180	TCTCCACCCA	6190	GGCCTGGAAT	6200	GTTTCCACCC	6210	AATGTCGAGC	6220	AGTGTGGTTT	6230	TGCAAGAGGA
6240	AGCAAAAAGC	6250	CTCTCCACCC	6260	AGGCCTGGAA	6270	TGTTTCCACC	6280	CAATGTCGAG	6290	CAAACCCCGC	6300	CCAGCGTCTT
6310	GTCATTGGCG	6320	AATTGGAACA	6330	CGCATATGCA	6340	GTCGGGGCGG	6350	CGCGGTCCCA	6360	GGTCCACTTC	6370	GCATATTAAAG
6380	GTGGCGCGTG	6390	TGGCCTCGAA	6400	CACCGAGCGA	6410	CCCTGCAGCC	6420	AATATGGGAT	6430	CGGCCATTGA	6440	ACAAGATGGA
6450	TTGCACGCAG	6460	GTTCTCCGGC	6470	CGCTTGGGTG	6480	GAGAGGCTAT	6490	TCGGCTATGA	6500	CTGGGCACAA	6510	CAGACAATCG
6520	GCTGCTCTGA	6530	TGCCGCCGTG	6540	TTCCGGCTGT	6550	CAGCGCAGGG	6560	GCGCCCGGTT	6570	CTTTTGTCA	6580	AGACCGACCT
6590	GTCCGGTGCC	6600	CTGAATGAAC	6610	TGCAGGTAAG	6620	TGCGGCCCGTC	6630	GATGGCCGAG	6640	GCGGCCTCGG	6650	CCTCTGCATA
6660	AATAAAAAAA	6670	ATTAGTCAGC	6680	CATGCATGGG	6690	GCGGAGAATG	6700	GGCGGAACTG	6710	GGCGGAGTTA	6720	GGGGCGGGAT
6730	GGGCGGAGTT	6740	AGGGCGGGGA	6750	CTATGGTTGC	6760	TGACTAATTG	6770	AGATGCATGC	6780	TTTGCATACT	6790	TCTGCCTGCT
6800	GGGGAGCCTG	6810	GGGACTTTCC	6820	ACACCTGGTT	6830	GCTGACTAAT	6840	TGAGATGCAT	6850	GCTTTGCATA	6860	CTTCTGCCTG

FIG. 10G

63/75

6870	6880	6890	6900	6910	6920	6930
CTGGGGAGCC	TGGGGACTTT	CCACACCCCTA	ACTGACACAC	ATTCACACAGA	ATTAATTTCCC	CTAGTTATTA
6940	6950	6960	6970	6980	6990	7000
ATAGTAATCA	ATTACGGGGT	CATTAGTTCA	TAGCCCATAT	ATGGAGTTCC	GCGTTACATA	ACTTACGGTA
7010	7020	7030	7040	7050	7060	7070
AATGGCCCGC	CTGGCTGACC	GCCCAACGAC	CCCCGCCCAT	TGACGTCAT	AATGACGTAT	GTTCCCATAG
7080	7090	7100	7110	7120	7130	7140
TAACGCCCAAT	AGGGACTTTC	CATTGACGTC	AATGGGTGGA	GTATTTACGG	TAAACTGCCC	ACTTGGCAGT
7150	7160	7170	7180	7190	7200	7210
ACATCAAGTG	TATCATATGC	CAAGTACGCC	CCCTATTGAC	GTCAATGACG	GTAATGGCC	CGCCTGGCAT
7220	7230	7240	7250	7260	7270	7280
TATGCCCAGT	ACATGACCCT	ATGGGACTTT	CCTACTTGCC	AGTACATCTA	CGTATTAGTC	ATCGCTATTA
7290	7300	7310	7320	7330	7340	7350
CCATGGTGAT	GCGGTTTTGG	CAGTACATCA	ATGGGCGTGG	ATAGCGGTTT	GACTCACGGG	GATTTCCAAG
7360	7370	7380	7390	7400	7410	7420
TCTCCACCCC	ATTGACGTCA	ATGGGAGTTT	GTTTTGGCAC	CAAAATCAAC	GGGACTTTCC	AAAATGTCGT
7430	7440	7450	7460	7470	7480	7490
AACAACCTCCG	CCCCATTGAC	GCAAATGGGC	GTAGGCGGTG	TACGGTGGGA	GGTCTATATA	AGCAGAGCTG
7500	7510	7520	7530	7540	7550	7560
GGTACGTGAA	CCGTCAGATC	GCCTGGAGAC	GCCATCACAG	ATCTCTCACC	ATGGACATGA	GGGTCCCCGC
7570	7580	7590	7600	7610	7620	7630
TCAGCTCCTG	GGGCTCCTTC	TGCTCTGGCT	CCCAGGTGCC	AGATGTGACA	TCCAGATGAC	CCAGTCTCCA
7640	7650	7660	7670	7680	7690	7700
TCTTCCCTGT	CTGCATCTGT	AGGGGACAGA	GTCACCATCA	CTTGACGGGC	AAGTCAGGAC	ATTAGGTATT
7710	7720	7730	7740	7750	7760	7770
ATTAAATTG	GTATCAGCAG	AAACCAGGAA	AAGCTCCTAA	GCTCCTGATC	TATGTTGCAT	CCAGTTTGCA
7780	7790	7800	7810	7820	7830	7840
AAGTGGGGTC	CCATCAAGGT	TCAGCGGCAG	TGGATCTGGG	ACAGAGTTCA	CTCTCACCGT	CAGCAGCCTG

FIG. 10H

64/75

7850	7860	7870	7880	7890	7900	7910
CAGCCTGAAG	ATTTTGGGAC	TTATTACTGT	CTACAGGTTT	ATAGTACCCC	TCGGACGTTT	GGCCAAGGGA
7920	7930	7940	7950	7960	7970	7980
CCAAGGTGGA	AATCAAACGT	ACGGTGGCTG	CACCATCTGT	CTTCATCTTC	CCGCCATCTG	ATGAGCAGTT
7990	8000	8010	8020	8030	8040	8050
GAAATCTGGA	ACTGCCCTCTG	TTGTGTGCCT	GCTGAATAAC	TTCTATCCCA	GAGAGGCCAA	AGTACAGTGG
8060	8070	8080	8090	8100	8110	8120
AAGGTGGATA	ACGCCCTCCA	ATCGGGTAAC	TCCCAGGAGA	GTGTCACAGA	GCAGGACAGC	AAGGACAGCA
8130	8140	8150	8160	8170	8180	8190
CCTACAGCCT	CAGCAGCACCC	CTGACGCTGA	GCAAAGCAGA	CTACGAGAAA	CACAAAGTCT	ACGCCTGCCA
8200	8210	8220	8230	8240	8250	8260
AGTCACCCAT	CAGGGCCTGA	GCTCGCCCGT	CACAAAGAGC	TTC AACAGGG	GAGAGTGTG	AATTCAGATC
8270	8280	8290	8300	8310	8320	8330
CGTTAACGGT	TACCAACTAC	CTAGACTGGA	TTCGTGACAA	CATGCGGCCG	TGATATCTAC	GTATGATCAG
8340	8350	8360	8370	8380	8390	8400
CCTCGACTGT	GCCTTCTAGT	TGCCAGCCAT	CTGTTGTTTG	CCCCCTCCCC	GTGCCCTTCT	TGACCCTGGA
8410	8420	8430	8440	8450	8460	8470
AGGTGCCACT	CCCACGTGCC	TTTCCTAATA	AAATGAGGAA	ATTGCATCGC	ATTGCTCTGAG	TAGGTGTCTAT
8480	8490	8500	8510	8520	8530	8540
TCTATTCTGG	GGGGTGGGGT	GGGGCAGGAC	AGCAAAGGGGG	AGGATTGGGA	AGACAATAGC	AGGCATGCTG
8550	8560	8570	8580	8590	8600	8610
GGGATGCCGGT	GGGCTCTATG	GCTTCTGAGG	CGGAAAGAAC	CAGCTGGGAC	TAGTCGCAAT	TGGGCGGAGT
8620	8630	8640	8650	8660	8670	8680
TAGGGGCGGG	ATGGCGGAG	TTAGGGCGGG	GGACTATGGT	GCTGACTAAT	TGAGATGCAT	GCTTTGCATA
8690	8700	8710	8720	8730	8740	8750
CTTCTGCCCTG	CTGGGGAGCC	TGGGGACTTT	CCACACCTGG	TTGCTGACTA	ATTGAGATGC	ATGCTTTGCA
8760	8770	8780	8790	8800	8810	8820
TACTTCTGCC	TGCTGGGGAG	CCTGGGGACT	TCCACACCC	TAACTGACAC	ACATTCCACA	GAATTAATTC

FIG. 10I

65/75

8830	CCCTAGTTAT	8840	TAATAGTAAT	8850	CAATTACGGG	8860	GTCATTAGTT	8870	CATAGCCCAT	8880	ATATGGAGTT	8890	CCGCGTTACA
8900	TAACTTACGG	8910	TAAATGGCCC	8920	GCCTGGCTGA	8930	CCGCCCAACG	8940	ACCCCCGCC	8950	ATTGACGTCA	8960	ATAATGACGT
8970	ATGTTCCCAT	8980	AGTAACGCCA	8990	ATAGGGACTT	9000	TCCATTGACG	9010	TCAATGGGTG	9020	GAGTATTTAC	9030	GGTAAACTGC
9040	CCACTTGGCA	9050	GTACATCAAG	9060	TGTATCATAT	9070	GCCAAGTACG	9080	CCCCCTATTG	9090	ACGTCAATGA	9100	CGGTAAATGG
9110	CCCCCTGGC	9120	ATTATGCCCA	9130	GTACATGACC	9140	TTATGGGACT	9150	TTCCTACTTG	9160	GCAGTACATC	9170	TACGTATTAG
9180	TCATCGCTGT	9190	TACCATGGTG	9200	ATGCGGTTTT	9210	GGCAGTACAT	9220	CAATGGGCGT	9230	GGATAGCGGT	9240	TTGACTCACG
9250	GGGATTTCCA	9260	AGTCTCCACC	9270	CCATTGACGT	9280	CAATGGGAGT	9290	TTGTTTTGGC	9300	ACCAAAATCA	9310	ACGGGACTTT
9320	CCAAAATGTC	9330	GTAACAACTC	9340	CGCCCCATTG	9350	ACGCAAATGG	9360	GCGGTAGGCG	9370	TGTACGGTGG	9380	GAGGTCTATA
9390	TAAGCAGAGC	9400	TGGGTACGTG	9410	AACCGTCAGA	9420	TCGCCCTGGAG	9430	ACGCCGTCTGA	9440	CATGGGTTGG	9450	AGCCTCATCT
9460	TGCTCTTCCCT	9470	TGTCGCTGTT	9480	GCTACGCGTG	9490	TCCTGTCCGA	9500	GGTGCAGCTG	9510	GTGGAGTCTG	9520	GGGGCCGGCTT
9530	GGCAAAGCCT	9540	GGGGGGTCCC	9550	TGAGACTCTC	9560	CTGCGCAGCC	9570	TCCGGGTTCA	9580	GGTTCACCCTT	9590	CAATAACTAC
9600	TACATGGACT	9610	GGGTCCGCCA	9620	GGCTCCAGGG	9630	CAGGGGCTGG	9640	AGTGGGTCTC	9650	ACGTATTAGT	9660	AGTAGTGGTG
9670	ATCCCACATG	9680	GTACGCAGAC	9690	TCCGTGAAGG	9700	GCAGATTAC	9710	CATCTCCAGA	9720	GAGAACGCCA	9730	AGAACACACT
9740	GTTTCTTCAA	9750	ATGAACAGCC	9760	TGAGAGCTGA	9770	GGACACGGCT	9780	GTCTATTACT	9790	GTGCGAGCTT	9800	GACTACAGGG

FIG. 10J

66/75

9810	9820	9830	9840	9850	9860	9870
TCTGACTCCCT	GGGGCCAGGG	AGTCCTGGTC	ACCGTCTCCT	CAGCTAGCAC	CAAGGGCCCCA	TCGGTCTTCC
9880	9890	9900	9910	9920	9930	9940
CCCTGGCACC	CTCCTCCAAG	AGCACCTCTG	GGGGCACACG	GGCCCTGGGC	TGCCCTGGTCA	AGGACTACTT
9950	9960	9970	9980	9990	10000	10010
CCCCGAACCG	GTGACGGTGT	CGTGGAATC	AGCGGCCCTG	ACCAGCGCGG	TGCACACCTT	CCCGGCTGTC
10020	10030	10040	10050	10060	10070	10080
CTACAGTCCT	CAGGACTCTA	CTCCCTCAGC	AGCGTGGTGA	CCGTGCCCTC	CAGCAGCTTG	GGCACCCAGA
10090	10100	10110	10120	10130	10140	10150
CCTACATCTG	CAACGTGAAT	CACAAGCCCA	GCAACACCAA	GGTGACAAAG	AAAGTTGAGC	CCAAATCTTG
10160	10170	10180	10190	10200	10210	10220
TGACAAAAT	CACACATGCC	CACCGTGCCC	AGCACCTGAA	CTCCTGGGGG	GACCGTCAGT	CTTCCCTCTC
10230	10240	10250	10260	10270	10280	10290
CCCCCAAAC	CCAAGGACAC	CCTCATGATC	TCCCGGACCC	CTGAGGTAC	ATGCGTGGTG	GTGGACGTGA
10300	10310	10320	10330	10340	10350	10360
GCCACGAAGA	CCCTGAGGTC	AAGTTCAACT	GGTACGTGGA	CGGCGTGGAG	GTGCATAATG	CCAAGACAAA
10370	10380	10390	10400	10410	10420	10430
GCCGCGGGAG	GAGCAGTACA	ACAGCACGTA	CCGTGTGTC	AGCGTCTCA	CCGTCTCTGCA	CCAGGACTGG
10440	10450	10460	10470	10480	10490	10500
CTGAATGGCA	AGGAGTACAA	GTGCAAGGTC	TCCAACAAAG	CCCTCCCAGC	CCCCATCGAG	AAAACCATCT
10510	10520	10530	10540	10550	10560	10570
CCAAAGCCAA	AGGGCAGCCC	CGAGAACCAC	AGGTGTACAC	CCTGCCCCCA	TCCCGGGATG	AGCTGACCAA
10580	10590	10600	10610	10620	10630	10640
GAACCAAGTC	AGCCTGACCT	GCCTGGTCAA	AGGCTTCTAT	CCCAGCGACA	TCGCCGTGGA	GTGGGAGAGC
10650	10660	10670	10680	10690	10700	10710
AATGGGCAGC	CGGAGAACAA	CTACAAGACC	ACGCCCTCCCG	TGCTGGACTC	CGACGGCTCC	TTCTTCCTCT
10720	10730	10740	10750	10760	10770	10780
ACAGCAAGCT	CACCGTGGAC	AAGAGCAGGT	GGCAGCAGGG	GAACGTCTTC	TCATGCTCCG	TGATGCATGA

FIG. 10K

10790	GGCTCTGCAC	10800	AACCACTACA	10810	CGCAGAAGAG	10820	CCTCTCCCTG	10830	TCTCCGGGTA	10840	AATGAGGATC	10850	CGTTAACGGT
10860	TACCAACTAC	10870	CTAGACTGGA	10880	TTCGTGACAA	10890	CATGCGGCCG	10900	TGATATCTAC	10910	GTATGATCAG	10920	CCTCGACTGT
10930	GCCTTCTAGT	10940	TGCCAGCCAT	10950	CTGTTGTTTGC	10960	CCCCTCCCCC	10970	GTGCCTTTCT	10980	TGACCCTGGA	10990	AGGTGCCACT
11000	CCCACTGTCC	11010	TTTCCTAATA	11020	AAATGAGGAA	11030	ATTGCATCGC	11040	ATTGCTGAG	11050	TAGGTGTCTAT	11060	TCTATTCTGG
11070	GGGTGGGGT	11080	GGGCAGGAC	11090	AGCAAGGGG	11100	AGGATTGGA	11110	AGACAATAGC	11120	AGGCATGCTG	11130	GGGATGCGGT
11140	GGGCTCTATG	11150	GCTTCTGAGG	11160	CGGAAAGAAC	11170	CAGCTGGGGC	11180	TCGACAGCAA	11190	CGCTAGGTCTG	11200	AGGCCGCTAC
11210	TAACTCTCTC	11220	CTCCCTCCTT	11230	TTTCTCTGAG	11240	GACGAGGCAG	11250	CGCGGCTATC	11260	GTGGCTGGCC	11270	ACGACGGGCG
11280	TTCCTTGCGC	11290	AGCTGTGCTC	11300	GACGTTGTCA	11310	CTGAAGCGGG	11320	AAGGGAAGTGG	11330	CTGCTATTGG	11340	GCGAAGTGCC
11350	GGGGCAGGAT	11360	CTCCTGTCTAT	11370	CTCACCTTGC	11380	TCCTGCCGAG	11390	AAAGTATCCA	11400	TCATGGCTGA	11410	TGCAATGCCG
11420	CGGCTGCATA	11430	CGCTTGATCC	11440	GGCTACCTGC	11450	CCATTGACC	11460	ACCAAGCGAA	11470	ACATCGCATC	11480	GAGCGAGCAC
11490	GTAAGCCGGT	11500	GGAAGCCGGT	11510	CTTGTCGATC	11520	AGGATGATCT	11530	GGACGAAGAG	11540	CATCAGGGGC	11550	TCGCGCCAGC
11560	CGAACTGTTT	11570	GCCAGGTAAG	11580	TGAGCTCCAA	11590	TTCAAGCTCT	11600	CGAGCTAGGG	11610	CGGCCAGCTA	11620	GTAGCTTTGC
11630	TTCTCAATTT	11640	CTTATTTGCA	11650	TAATGAGAAA	11660	AAAAGGAAAA	11670	TTAATTTTAA	11680	CACCAATTCA	11690	GTAGTTGATT
11700	GAGCAAATGC	11710	GTTGCCAAAA	11720	AGGATGCTTT	11730	AGAGACAGTG	11740	TTCTCTGCAC	11750	AGATAAGGAC	11760	AAACATTATT

FIG. 10L

68/75

11770	CAGAGGGAGT	11780	ACCCAGAGCT	11790	GAGACTCCTA	11800	AGCCAGTGAG	11810	TGGCACAGCA	11820	TCCAGGGAGA	11830	AATATGCTTG
11840	TCATCACCGA	11850	AGCCTGATTC	11860	CGTAGAGCCA	11870	CACCCCTGGTA	11880	AGGGCCAATC	11890	TGCTCACACA	11900	GGATAGAGAG
11910	GGCAGGAGCC	11920	AGGCAGAGC	11930	ATATAAGGTG	11940	AGGTAGGATC	11950	AGTTGCTCCT	11960	CACATTTGCT	11970	TCTGACATAG
11980	TTGTGTTGGG	11990	AGCTTGGATA	12000	GCTTGGGGGG	12010	GGGACAGCTC	12020	AGGGCTGCCA	12030	TTTCGGCGCA	12040	AACTTGACGG
12050	CAATCCTAGC	12060	GTGAAGGCTG	12070	GTAGGATTTT	12080	ATCCCCGCTG	12090	CCATCATGGT	12100	TCGACCATTG	12110	AACTGCATCG
12120	TCGCCGTGTC	12130	CCAAAATATG	12140	GGGATTGGCA	12150	AGAACGGAGA	12160	CCTACCCCTGG	12170	CCTCCGCTCA	12180	GGAACGAGTT
12190	CAAGTACTTC	12200	CAAAGAATGA	12210	CCACAACCTC	12220	TTCAGTGGAA	12230	GGTAAACAGA	12240	ATCTGGTGAT	12250	TATGGGTAGG
12260	AAAACCTGGT	12270	TCTCCATTCC	12280	TGAGAAGAAAT	12290	CGACCTTTAA	12300	AGGACAGAAT	12310	TAATATAGTT	12320	CTCAGTAGAG
12330	AACTCAAAGA	12340	ACCACCACGA	12350	GGAGCTCATT	12360	TTCTTGCCAA	12370	AAGTTTGAT	12380	GATGCCCTAA	12390	CGTAGGCGCG
12400	CCATTAGAC	12410	TTATTGAACA	12420	ACCGGAATTG	12430	GCAAGTAAAG	12440	TAGACATGGT	12450	TTGGATAGTC	12460	GGAGGCAGTT
12470	CTGTTTACCA	12480	GGAAGCCATG	12490	AATCAACCAG	12500	GCAACCTCAG	12510	ACTCTTTGTG	12520	ACAAGGATCA	12530	TGCAGGAATT
12540	TGAAAGTGAC	12550	ACGTTTTTCC	12560	CAGAAATTGA	12570	TTTGGGGAAA	12580	TATAAACTTC	12590	TCCCAGAATA	12600	CCCAGGCGTC
12610	CTCTCTGAGG	12620	TCAAGGAGGA	12630	AAAAGGCATC	12640	AAGTATAAGT	12650	TTGAAGTCTA	12660	CGAGAAGAAA	12670	GACTAACAGG
12680	AAGATGCTTT	12690	CAAGTTCTCT	12700	GCTCCCCCTC	12710	TAAAGCTATG	12720	CATTTTATA	12730	AGACCATGGG	12740	ACTTTTGCTG

FIG. 10M

69/75

12750	12760	12770	12780	12790	12800	12810
GCTTAGATC	AGCCTCGACT	GTGCCCTTCTA	GTTGCCAGCC	ATCTGTTGTT	TGCCCCCTCCC	CCGTGCCTTC
12820	12830	12840	12850	12860	12870	12880
CTTGACCCCTG	GAAGGTGCCA	CTCCCACTGT	CCTTTCTTAA	TAAATGAGG	AAATTGCATC	GCAATTGCTG
12890	12900	12910	12920	12930	12940	12950
AGTAGGTGTC	ATTCTATTCT	GGGGGGTGGG	GTGGGGCAGG	ACAGCAAGGG	GGAGGATTGG	GAAGACAATA
12960	12970	12980	12990	13000	13010	13020
GCAGGCATGC	TGGGGATGCG	GTGGGCTCTA	TGGCTTCTGA	GGCGGAAAGA	ACCAGCTGGG	GCTCGAAGCG
13030	13040	13050	13060	13070	13080	13090
GCCGCCCATT	TCGCTGGTGG	TCAGATGCGG	GATGGCGTGG	GACGCGGCGG	GGAGCGTCAC	ACTGAGGTTT
13100	13110	13120	13130	13140	13150	13160
TCCGCCAGAC	GCCACTGCTG	CCAGGCGCTG	ATGTGCCCGG	CTTCTGACCA	TGCGGTGCGG	TTCGGTTGCA
13170	13180	13190	13200	13210	13220	13230
CTACGCGTAC	TGTGAGCCAG	AGTTGCCCGG	CGCTCTCCGG	CTGCGGTAGT	TCAGGCAGTT	CAATCAACTG
13240	13250	13260	13270	13280	13290	13300
TTTACCTTGT	GGAGCGACAT	CCAGAGGCAC	TTCACGCTT	GCCAGCGGCT	TACCATCCAG	CGCCACCATC
13310	13320	13330	13340	13350	13360	13370
CAGTGCAGGA	GCTCGTTATC	GCTATGACGG	AACAGGTATT	CGCTGTGCAC	TTCGATGTT	TGCCCGGATA
13380	13390	13400	13410	13420	13430	13440
AACGGAACTG	GAAAACTGC	TGCTGGTGT	TTGCTTCCGT	CAGCGCTGGA	TGCGGCGTGC	GGTCGGCAAA
13450	13460	13470	13480	13490	13500	13510
GACCAGACCG	TTTATACAGA	ACTGGCGATC	CGTTCGGCTA	TCGCCAAAAT	CACCGCCGTA	AGCCGACCAC
13520	13530	13540	13550	13560	13570	13580
GGGTTGCCGT	TTTATCATA	TTTAATCAGC	GACTGATCCA	CCAGTCCCA	GACGAAGCCG	CCCTGTAAAC
13590	13600	13610	13620	13630	13640	13650
GGGGATACTG	ACGAAACGCC	TGCCAGTATT	TAGCGAAACC	GCCAAAGACTG	TTACCCATCG	CGTGGGCGTA
13660	13670	13680	13690	13700	13710	13720
TTCGCAAAGG	ATCAGCGGGC	GCGTCTCTCC	AGGTAGCGAA	AGCCATTTTT	TGATGGACCA	TTTCGGCACA

FIG. 10N

13730	13740	13750	13760	13770	13780	13790
GCCGGGAAGG	GCTGGTCTTC	ATCCACGCGC	GGGTACATCG	GGCAATAAT	ATCGGTGGCC	GTGGTGTCCG
13800	13810	13820	13830	13840	13850	13860
CTCCGCCGCC	TTCATACTGC	ACCGGGCGGG	AAGGATCGAC	AGATTGATC	CAGCGATACA	GCGCGTCGTG
13870	13880	13890	13900	13910	13920	13930
ATTAGCGCCG	TGGCCTGATT	CATTCCCCAG	CGACCAGATG	ATCACACTCG	GGTGATTACG	ATCGCGCTGC
13940	13950	13960	13970	13980	13990	14000
ACCATTCGGG	TTACGCGTTC	GCTCATCGCC	GGTAGCCAGC	GCGGATCATC	GGTCAGACGA	TTCATTGGCA
14010	14020	14030	14040	14050	14060	14070
CCATGCCCGT	GGTTTCAATA	TTGGCTTCAT	CCACCACATA	CAGGCCGTAG	CGGTGCGACA	GCGGTGTACCA
14080	14090	14100	14110	14120	14130	14140
CAGCGGATGG	TTCGGATAAT	GCGAACAGCG	CACGGCGTTA	AAGTTGTTCT	GCTTCATCAG	CAGGATATCC
14150	14160	14170	14180	14190	14200	14210
TGCACCATCG	TCTGCTCATC	CATGACCTGA	CCATGCAGAG	GATGATGCTC	GTGACGGTTA	ACGCCCTCGAA
14220	14230	14240	14250	14260	14270	14280
TCAGCAACGG	CTTGCCGTTT	AGCAGCAGCA	GACCATTTTC	AATCCGCACC	TCGCGGAAAC	CGACATCGCA
14290	14300	14310	14320	14330	14340	14350
GGCTTCTGCT	TCAATCAGCG	TGCCGTCGGC	GGTGTGCAGT	TCAACCACCG	CACGATAGAG	ATTCCGGATT
14360	14370	14380	14390	14400	14410	14420
TCGGCGCTCC	ACAGTTTCGG	GTTTTGACG	TTCAGACGTA	GTGTGACGCG	ATCGGCATAA	CCACCACGCT
14430	14440	14450	14460	14470	14480	14490
CATCGATAAT	TTCACCGCCG	AAAGGCGCGG	TGCCGCTGGC	GACCTGCGTT	TCACCCCTGCC	ATAAAGAAAC
14500	14510	14520	14530	14540	14550	14560
TGTTACCCGT	AGGTAGTCAC	GCAACTCGCC	GCACATCTGA	ACTTCAGCCT	CCAGTACAGC	GCGGCTGAAA
14570	14580	14590	14600	14610	14620	14630
TCATCATTA	AGCGAGTGGC	AACATGGAAA	TCGCTGATTT	GTGTAGTCGG	TTTATGCAGC	AACGAGACGT
14640	14650	14660	14670	14680	14690	14700
CACGGAAAA	GCCGCTCATC	CGCCACATAT	CCTGATCTTC	CAGATAACTG	CCGTCACTCC	AGCGCAGCAC

FIG. 10P

71/75

14710	CATCACC	14720	AGGGG	14730	CTCCG	14740	TAAAA	14750	CTCAG	14760	ATTCA	14770	CAAAC
14780	TCCTGG	14790	AACCG	14800	GGCCC	14810	CACCAC	14820	GAAAC	14830	GTAAAC	14840	TCAAAA
14850	TTCCG	14860	GCCTTC	14870	AGCCAG	14880	CATCAAC	14890	AAATGT	14900	GAGTAAC	14910	CCGTCG
14920	CTCCG	14930	ACAAAC	14940	GATTG	14950	AATGG	14960	GTCAC	14970	TGTAG	14980	CGCATC
14990	CCGTGC	15000	GCCAG	15010	GGGAC	15020	ACAGT	15030	CCTCA	15040	ATCGC	15050	AGCCAG
15060	CCGGC	15070	TTCTG	15080	GGAAAC	15090	GCAAG	15100	TTCGC	15110	AGGCT	15120	ACTGT
15130	AGGGC	15140	GTGCG	15150	CTTCG	15160	ACGCC	15170	GCGAA	15180	GATGT	15190	AAGGC
15200	AGTTG	15210	CGCCAG	15220	TTCCC	15230	CGACG	15240	AAACG	15250	ATCCG	15260	GGGCT
15270	GAAGC	15280	ACCTT	15290	GTGCAG	15300	CGGCG	15310	GCCGT	15320	ACAA	15330	GCGAA
15340	TAAAC	15350	CAAAT	15360	CGGCG	15370	GCCGC	15380	CCTTC	15390	TGCC	15400	TCCAG
15410	CCACC	15420	CACCAC	15430	GATGT	15440	TTGCC	15450	CTCCA	15460	GCCG	15470	CCTCA
15480	CTGCAC	15490	AGACG	15500	CAACA	15510	TGGA	15520	TAGAA	15530	AAAA	15540	CTCGT
15550	ACCGGT	15560	CCAAA	15570	CGCCC	15580	CAGTA	15590	GTTG	15600	CGACT	15610	GCCTA
15620	ACATTT	15630	CTAAC	15640	GTCTG	15650	CCGCC	15660	CTCCG	15670	GCCTC	15680	CCAGC

FIG. 10Q

CTGGCGCTCC	15690	ACCGATGGTA	15700	GATTTATCAT	15710	CAGCTCCACC	15720	ACCGCCGCCA	15730	TTAGTAGATT	15740	TGCCGTCTGA	15750
AATGTTACCA	15760	CCGCCCTGCAC	15770	CATCGCTTTC	15780	TAACGTGTTG	15790	TCTGAATTAA	15800	AATCGGGCAC	15810	AGTTAGATTG	15820
AAACCCGCCC	15830	AAAAACGCCC	15840	GCAATCAGAA	15850	ATAATTCCAA	15860	AAAGCTCAAC	15870	TACAAATTTG	15880	ATCGCGGACG	15890
TGTTAGCCGA	15900	CACAAATTAAT	15910	AGGCGTCTGT	15920	TGGCTATGGC	15930	AAAATCGTCT	15940	TCGGAAGCAA	15950	CTTCTAACGA	15960
CGAGGGTTGG	15970	GACGACGACG	15980	ATAATCGGCC	15990	TAATAAGCT	16000	AACACGCCCG	16010	ATGTTAAATA	16020	TGTCCAAGCT	16030
ACTAGTGTA	16040	CCGCTTGGA	16050	GAACATATCC	16060	ATCGCGTCCG	16070	CCATCTCCAG	16080	CAGCCGCACG	16090	CGCGCATCT	16100
CGGGCAGCGT	16110	TGGGTCTCTG	16120	CCACGGGTGC	16130	GCATGATCGT	16140	GCTCCTGTCTG	16150	TTGAGGACCC	16160	GGCTAGGCTG	16170
GCGGGGTTGC	16180	CTTACTGGTT	16190	AGCAGAATGA	16200	ATCACCATA	16210	CGCGAGCGAA	16220	CGTGAAGCGA	16230	CTGCTGCTGC	16240
AAAACGTCTG	16250	CGACCTGAGC	16260	AACAACATGA	16270	ATGGTCTTCG	16280	GTTTCCGTGT	16290	TTCGTAAAGT	16300	CTGGAAACGC	16310
GGAAGTCAGC	16320	GCCCTGCACC	16330	ATTATGTTCC	16340	GGATCTGCAT	16350	CGCAGGATGC	16360	TGCTGGCTAC	16370	CCTGTGGAAC	16380
ACCTACATCT	16390	GTATTAACGA	16400	AGCGCTGGCA	16410	TTGACCCCTGA	16420	GTGATTTTTC	16430	TCTGGTCCCG	16440	CCGCATCCAT	16450
ACCGCCAGTT	16460	GTTTACCCTC	16470	ACAACGTTCC	16480	AGTAACCCGGG	16490	CATGTTTCATC	16500	ATCAGTAAAC	16510	CGTATCGTGA	16520
GCATCCTCTC	16530	TCGTTTTCATC	16540	GGTATCATTA	16550	CCCCCATGAA	16560	CAGAAATCCC	16570	CCTTACACGG	16580	AGGCATCAGT	16590
GACCAAACAG	16600	GAAAAAACCG	16610	CCCTTAACAT	16620	GGCCCGCTTT	16630	ATCAGAAAGCC	16640	AGACATTAAAC	16650	GCTTCTGGAG	16660

FIG. 10R

73/75

16670	16680	16690	16700	16710	16720	16730
AAACTCAACG	AGCTGGACGC	GGATGAACAG	GCAGACATCT	GTGAATCGCT	TCACGACCAC	GCTGATGAGC
16740	16750	16760	16770	16780	16790	16800
TTTACCGCAG	CTGCCTCGCG	CGTTTCGGTG	ATGACGGTGA	AAACCTCTGA	CACATGCAGC	TCCCGGAGAC
16810	16820	16830	16840	16850	16860	16870
GGTCACAGCT	TGTCTGTAAG	CGGATGCCCGG	GAGCAGACAA	GCCCCTCAGG	GGCGTCAGC	GGGTGTTGGC
16880	16890	16900	16910	16920	16930	16940
GGGTGTCGGG	GCGCAGCCAT	GACCCAGTCA	CGTAGCGATA	GCGGAGTGTA	TACTGGCTTA	ACTATGCGGC
16950	16960	16970	16980	16990	17000	17010
ATCAGAGCAG	ATTGTAAGTGA	GAGTGCACCA	TATGCGGTGT	GAAATACCGC	ACAGATGCGT	AAGGAGAAAA
17020	17030	17040	17050	17060	17070	17080
TACCGCATCA	GGCGCTCTTC	CGCTTCCTCG	CTCACTGACT	CGCTGCGCTC	GGTCGTTCCG	CTGCGGCGAG
17090	17100	17110	17120	17130	17140	17150
CGGTATCAGC	TCACTCAAAG	GCGGTAATAC	GTTATCCAC	AGAATCAGGG	GATAACGCAG	GAAAGAACAT
17160	17170	17180	17190	17200	17210	17220
GTGAGCAAAA	GGCCAGCAAA	AGGCCAGGAA	CCGTAAAAAG	GCCGCGTTGC	TGGCGTTTTT	CCATAGGCTC
17230	17240	17250	17260	17270	17280	17290
CGCCCCCCTG	ACGAGCATCA	CAAAAATCGA	CGCTCAAGTC	AGAGGTGGCG	AAACCCGACA	GGAATAAAA
17300	17310	17320	17330	17340	17350	17360
GATACCAGGC	GTTTCCCCCT	GGAAGCTCCC	TCGTGCGCTC	TCCTGTTCCG	ACCCTGCCGC	TTACCCGATA
17370	17380	17390	17400	17410	17420	17430
CCTGTCCGCC	TTTCTCCCTT	CGGGAAGCGT	GGCGCTTCT	CATAGCTCAC	GCTGTAGGTA	TCTCAGTTCC
17440	17450	17460	17470	17480	17490	17500
GTGTAGGTCG	TTCGCTCCAA	GCTGGGCTGT	GTGCACGAAC	CCCCCGTTCA	GCCCGACCCG	TGCGCCTTAT
17510	17520	17530	17540	17550	17560	17570
CCGGTAACTA	TCGTCTTGAG	TCCAACCCCG	TAAGACACGA	CTTATCGCCA	CTGGCAGCAG	CCACTGGTAA
17580	17590	17600	17610	17620	17630	17640
CAGGATTAGC	AGAGCGAGGT	ATGTAGGCGG	TGCTACAGAG	TTCTTGAAAGT	GGTGGCCTAA	CTACGGGCTAC

FIG. 10S

74/75

17650	17660	17670	17680	17690	17700	17710
ACTAGAAGGA	CAGTATTTGG	TATCTGCGCT	CTGCTGAAGC	CAGTTACCTT	CGGAAAAAGA	GTTGGTAGCT
17720	17730	17740	17750	17760	17770	17780
CTTGATCCGG	CAACAAACC	ACCGCTGGTA	GCGGTGTTT	TTTTGTTTC	AAGCAGCAGA	TTACGCGCAG
17790	17800	17810	17820	17830	17840	17850
AAAAAAGGA	TCTCAAGAAG	ATCCTTTGAT	CTTTTCTACG	GGGTCTGACG	CTCAGTGGAA	CGAAAACTCA
17860	17870	17880	17890	17900	17910	17920
CGTTAAGGGA	TTTTGGTCAT	GAGATTATCA	AAAAGGATCT	TCACCTAGAT	CCTTTTAAAT	TAAAAATGAA
17930	17940	17950	17960	17970	17980	17990
GTTTTAAATC	AATCTAAAGT	ATATATGAGT	AAACTTGGTC	TGACAGTTAC	CAATGCTTAA	TCAGTGAGGC
18000	18010	18020	18030	18040	18050	18060
ACCTATCTCA	GCGATCTGTC	TATTTCTGTC	ATCCATAGTT	GCCTGACTCC	CCGTCGTGTA	GATAACTACG
18070	18080	18090	18100	18110	18120	18130
ATACGGGAGG	GCTTACCATC	TGGCCCCAGT	GCTGCAATGA	TACCGCGAGA	CCCACGCTCA	CCGGCTCCAG
18140	18150	18160	18170	18180	18190	18200
ATTTATCAGC	AATAAACCAG	CCAGCCCGAA	GGGCCGAGCG	CAGAAGTGGT	CCTGCAACTT	TATCCGCTC
18210	18220	18230	18240	18250	18260	18270
CATCCAGTCT	ATTAATTGTT	GCCGGGAAGC	TAGAGTAAGT	AGTTCGCCAG	TTAATAGTTT	GCGCAACGTT
18280	18290	18300	18310	18320	18330	18340
GTTGCCATTG	CTGCAGGCAT	CGTGGTGTCA	CGCTCGTCGT	TTGGTATGGC	TTCAATTCAGC	TCCGGTTCCC
18350	18360	18370	18380	18390	18400	18410
AACGATCAAG	GCGAGTTACA	TGATCCCCCA	TGTTGTGCAA	AAAAGCGGTT	AGCTCCTTCG	GTCTCTCCGAT
18420	18430	18440	18450	18460	18470	18480
CGTTGTGAGA	AGTAAGTTGG	CCGCAGTGTT	ATCACTCATG	GTTATGGCAG	CACTGCATAA	TTCTCTTACT
18490	18500	18510	18520	18530	18540	18550
GTCATGCCAT	CCGTAAGATG	CTTTTCTGTG	ACTGGTGAGT	ACTCAACCAA	GTCATTCTGA	GAATAGTGTA
18560	18570	18580	18590	18600	18610	18620
TGCGGCGACC	GAGTTGCTCT	TGCCCGGCGT	CAACACGGGA	TAATACCGCG	CCACATAGCA	GAACTTTAAA

FIG. 10T

75/75

18630	18640	18650	18660	18670	18680	18690
AGTGCTCATC	ATTGGAAAC	GTTCTTCGGG	GCGAAACTC	TCAAGGATCT	TACCGCTGTT	GAGATCCAGT
18700	18710	18720	18730	18740	18750	18760
TCGATGTAAC	CCACTCGTGC	ACCCAACGA	TCTTCAGCAT	CTTTTACTTT	CACCAGCGTT	TCTGGGTGAG
18770	18780	18790	18800	18810	18820	18830
CAAAAACAGG	AAGGCAAAAT	GCCGCAAAA	AGGGAATAAG	GGCGACACGG	AAATGTTGAA	TACTCATACT
18840	18850	18860	18870	18880	18890	18900
CTTCCTTTTT	CAATATTATT	GAAGCATTTA	TCAGGGTTAT	TGTCTCATGA	GCGGATACAT	ATTTGAATGT
18910	18920	18930	18940	18950	18960	18970
ATTTAGAAAA	ATAACAAAT	AGGGGTTCCG	CGCACATTTC	CCCGAAAAGT	GCCACCTGAC	GTCTAAGAAA
18980	18990	19000	19010	19020	19030	19040
CCATTATTAT	CATGACATTA	ACCTATAAAA	ATAGGCGTAT	CACGAGGCC	TTTCGTCTTC	AAGAA . . .
19050	19060	19070	19080			

FIG. 10U

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/03935

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/85 C12Q1/68 C12N5/10 C12N9/12  
 C12N15/13 C07K16/28 C12N15/12 C07K14/705 G01N33/53  
 C12N15/62 C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 11523 A (IDEC PHARMACEUTICALS CORPORATION (US); REFF MITCHELL E. (US)) 26 May 1994 cited in the application see abstract see page 9, line 21 - page 10, line 29 see page 41, line 19 - page 42, line 19; figure 6	1, 4-8, 11, 12, 25-29, 31, 32
A	US 5 464 764 A (CAPECCHI MARIO R. AND KIRK THOMAS R.) 7 November 1995 see abstract see column 13, line 32 - column 14, line 5	1
A	WO 94 05784 A (UNITED STATES AMERICA REPRESENTED BY THE SECRETARY US DPT. AGRICULTURE) 17 March 1994 see abstract	1
	--- -/-	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

23 July 1998

Date of mailing of the international search report

05/08/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Macchia, G

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of documents, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 24642 A (TSI CORPORATION (US)) 9 December 1993 see abstract	1
A	----- BARNETT R.S. ET AL.: "Antibody production in chinese hamster ovary cells using an impaired selectable marker" ACS SYMPOSIUM SERIES: ANTIBODY EXPRESSION AND ENGINEERING, vol. 604, 1995, pages 27-40, XP002072464 -----	

## INTERNATIONAL SEARCH REPORT

I. International Application No

PCT/US 98/03935

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9411523 A	26-05-1994	AU 682481 B	09-10-1997
		AU 5613294 A	08-06-1994
		CA 2149326 A	26-05-1994
		DE 669986 T	10-10-1996
		EP 0669986 A	06-09-1995
		ES 2088838 T	01-10-1996
		JP 8503138 T	09-04-1996
		US 5648267 A	15-07-1997
		US 5733779 A	31-03-1998
US 5464764 A	07-11-1995	US 5487992 A	30-01-1996
		US 5627059 A	06-05-1997
		US 5631153 A	20-05-1997
WO 9405784 A	17-03-1994	AU 4839493 A	29-03-1994
		MX 9305183 A	31-05-1994
WO 9324642 A	09-12-1993	AU 4401993 A	30-12-1993